

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
18 August 2005 (18.08.2005)

PCT

(10) International Publication Number
WO 2005/074933 A1

(51) International Patent Classification⁷: A61K 31/445,
C07D 401/04

[JP/US]; 3134 Bellaire Boulevard, Houston, TX 77025
(US). ASADA, Shinichi [JP/JP]; 3-21-5-202 Karasaki,
Otsu, Shiga 520-0106 (JP).

(21) International Application Number:
PCT/US2005/003349

(74) Agents: BAKKER, Jila et al.; Fulbright & Jaworski
L.L.P., Suite 5100, 1301 McKinney, Houston, TX
77010-3095 (US).

(22) International Filing Date: 28 January 2005 (28.01.2005)

(25) Filing Language: English

(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(26) Publication Language: English

(30) Priority Data:
10/770,303 2 February 2004 (02.02.2004) US

(71) Applicant (*for all designated States except US*): BAYLOR
COLLEGE OF MEDICINE [US/US]; One Baylor Plaza,
Suite 106A, Houston, TX 77030 (US).

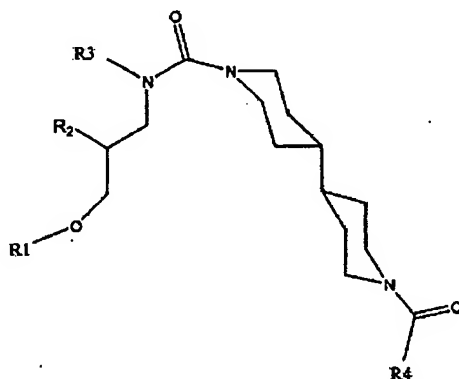
(72) Inventors; and

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,

(75) Inventors/Applicants (*for US only*): UESUGI, Motonari

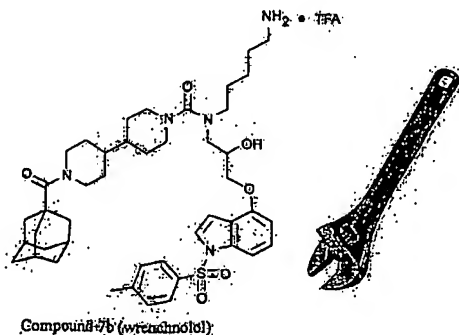
[Continued on next page]

(54) Title: SMALL MOLECULE INHIBITORS OF HER2 EXPRESSION



(57) Abstract: Peptide mimetic small molecule inhibitors of
Sur-2 are provided having the general formula (I).

A





GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

SMALL MOLECULE INHIBITORS OF HER2 EXPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/380,481, which was filed May 14, 2002, and which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING SPONSORED RESEARCH OR DEVELOPMENT

[0002] This research was supported by grant number Q-1490 from The Welch Foundation (Houston, TX).

TECHNICAL FIELD

[0003] The field of the invention relates to inhibitors of Her2 expression. The field of the invention also relates to peptide mimetic inhibitors of Sur-2, a Ras-linked subunit of the human mediator complex, and methods of their use the treatment of breast cancer in mammals.

BACKGROUND OF THE INVENTION

[0004] The proto-oncogene *HER2/neu* (*C-erbB-2*) encodes a transmembrane tyrosine kinase growth factor receptor. The name for the HER2 protein is derived from "*Human Epidermal growth factor Receptor*," as it features substantial homology with the epidermal growth factor receptor (EGFR). Overexpression of this transmembrane receptor has been found in ~30% of breast cancers (Slamon *et al.*, 1987), amounting to as many as 60-thousand cases a year in the United States. To date, a wide variety of clinical studies, including more than 15,000 patients, have evaluated the relationship between *HER2/neu* abnormalities and breast cancer outcome, including more than 47 publications concerning the association of gene and/or protein abnormalities with prognosis (Ross *et al.*, 1999). These studies clearly indicate that HER2 protein overexpression is associated with an adverse outcome in breast cancer.

[0005] Overexpression of the oncogene *Her2 (neu/ErbB2)* has been found in ~30% of breast tumors (Slamon *et al.*, 1987; Ross *et al.*, 1999). It is unclear why overexpression of this transmembrane receptor occurs in breast cancers, but patients who have Her2 excesses typically

have more aggressive disease with enhanced metastasis and increased resistance to chemotherapy. Monoclonal antibodies against the Her2 protein have been successful in treating these Her2-positive patients. A humanized antibody called Herceptin® has demonstrated tumor inhibitory and chemosensitizing effects in clinical studies and is the only drug that FDA has approved for treatment of *Her2*-overexpressing breast tumors (Drebin *et al.*, 1986; Drebin *et al.*, 1985). The clinical success of the Her2-antibody therapy was an excellent example of the translation of basic cancer biology into clinical cancer treatment. However, the antibody therapy alone may not be ideal for therapeutic intervention of *Her2*-overexpressing breast cancers. In theory, downregulation of *Her2* may be accomplished efficiently by inhibiting the expression of the *Her2* gene rather than targeting elevated levels of the Her2 proteins that are already overexpressed. By analogy with treatments for AIDS, cocktails of drugs — each with a different mechanisms of action — might also be more effective at achieving complete remission of breast tumors. Thus, discovering a means to provide external control over *Her2* expression, particularly through small organic molecules, remains appealing.

[0006] The overexpression of *Her2* can be accomplished through a combination of two different mechanisms: gene duplication and increased transcription. In both cases, excess *Her2* mRNA is produced, and this process can be controlled at the level of gene transcription. In search of transcription factors that activate the *Her2* gene in breast cancers, Chang *et al.* and others have identified the Ets factor ESX (epithelial-restricted with serine box) (Chang *et al.*, 1997). ESX is overexpressed in *Her2*-overexpressing breast cancer cells, and it binds and strongly transactivates the promoter of the *Her2* gene. Deletion of the ESX-binding element from the *Her2* promoter completely abolishes its activation, suggesting the importance of ESX in activating the *Her2* gene in cells (Scott *et al.*, 1994). The expression of ESX is epithelial specific and high in mammalian gland, trachea, prostate, and stomach (Andreoli *et al.*, 1997). ESX may be a unique transcription factor that controls expression of *Her2* in these tissues.

[0007] Current drug therapy addresses only about 500 molecular targets. Cell membrane receptors and enzymes account for ~80% of all current drug targets, and there are few drug targets in the nucleus except nuclear receptors (and DNA in the case of cancer chemotherapy) (Drews *et al.*, 2000). Discovering new molecular targets in the nucleus would extend the scope of drug targets and might provide alternative therapeutic strategies to treat major human diseases. For instance, recent discovery of histone deacetylases as a potential

target for cancer therapy had tremendous impacts on the drug discovery research (Kwon *et al.*, 1998; Hassing *et al.*, 1997; Taunton *et al.*, 1996; Lin *et al.*, 1998). The potentiality of transcriptional co-activators as a drug target has never been explored due to the lack of expected therapeutic phenotypes. A subset of co-activators, although not all, may serve as a target for pharmaceutical intervention.

[0008] Complete analysis of the human genome is anticipated to produce an unprecedented number of potential drug targets. Among these genomic pseudo-targets, the “relatively easy” targets such as GPCRs or enzymes will certainly be an immediate focus in pharmaceutical industries. However, more challenging genomic targets including protein-protein interactions need to be assessed for a leap of pharmaceutical development in the future.

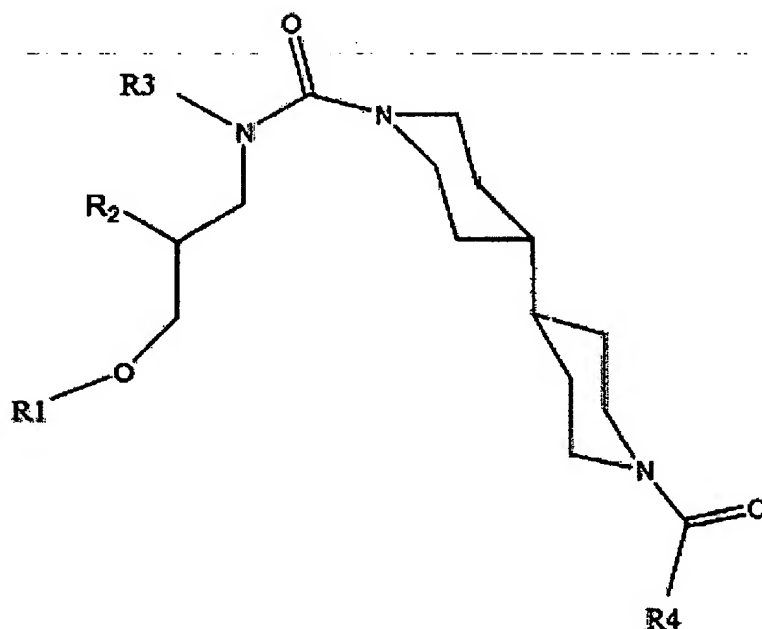
[0009] Protein-protein interactions are harder to target by small organic molecules than enzymes or nuclear hormone receptors. Protein-protein binding typically occurs over a relatively large surface area, and the binding surfaces between two proteins tend to be flat and often lack in pockets that might provide binding sites suited for small organic molecules. Nevertheless, protein-protein interfaces vary widely in nature from one to another, and some are likely to present better druggability than others. A good example is the interaction between the somatostatin receptor and β -turn peptide ligands.

[0010] Recent studies suggest that the protein-protein interactions that are mediated by short α -helical segments of proteins are similarly tractable to inhibition by small nonpeptidic molecules. Helical peptide segments of proteins are responsible for a number of biologically important protein associations in the fields of signal transduction and gene transcription. The interaction between the two cancer-linked nuclear proteins, ESX (an epithelial-specific transcription factor) and Sur-2/ DRIP130 (a Ras-linked subunit of the human mediator complex) is required for the overexpression of the *Her2* oncogene in malignant breast cancer cells and thus serves as a potential therapeutic target for Her2-positive breast cancers amounting to 60,000 cases per year in the United States. The interaction is mediated by one face of an eight-amino acid-helical region in the transcriptional activation domain of ESX (SEQ ID NO: 1 Ser-Trp- Ile- Ile-Glu-Leu-Leu-Glu), and the tryptophan residue in the hydrophobic face of the helix makes a unique contribution to the specificity of the interaction. The relatively small size of the interface and the importance of the tryptophan residue suggested the existence of small-molecule

inhibitors in a chemical library enriched in the structural families of indole, benzimidazole, and benzodiazepin — indole-mimicking π -electron-rich pharmacores found in bioavailable drugs.

BRIEF SUMMARY OF THE INVENTION

[0011] An object of the present invention is to provide small molecule Her2 expression inhibitors. In an embodiment of the invention, the Her2 expression inhibitors are peptide mimetics. In another embodiment of the invention, the Her2 expression inhibitors are α -helix mimetics of ESX. In an embodiment of the invention, the Her2 expression inhibitors are α -helix mimetics of SEQ ID NO:1. An embodiment of the invention is a compound of the formula:

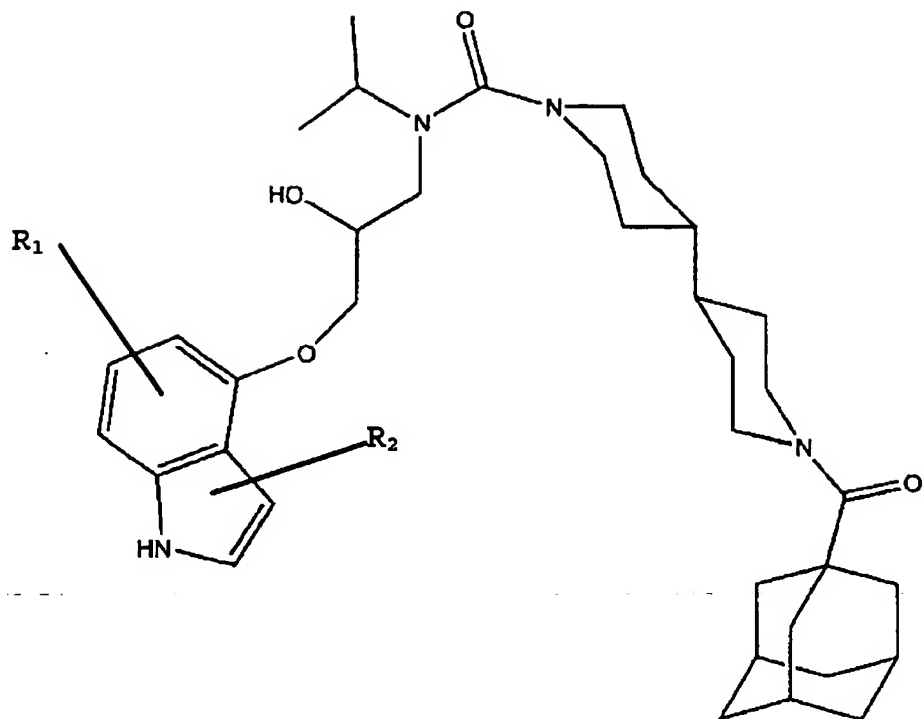


wherein R₁ is an indole, indole derivative, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, sulfinyl, sulfonyl, carbonyl, cyano, nitro, halo, or

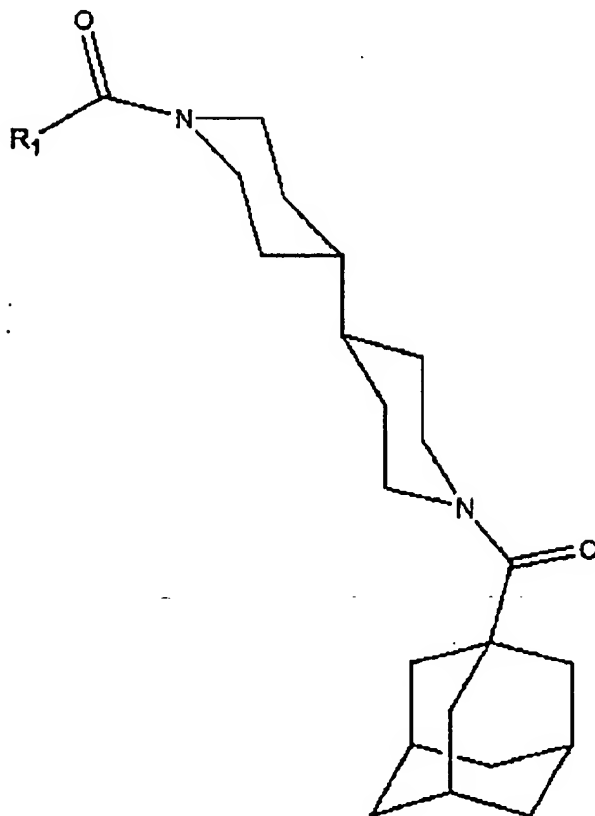
amino; wherein R₂ is a hydrogen, hydroxy, nitrate, halo, loweralkyl, or carbonyl; wherein R₃ is a halo, loweralkyl, carbonyl, aryl, aralkyl, or heterocycle; and wherein R₄ is an adamantane, adamantane derivative, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, sulfinyl, sulfonyl, S-sulfonamido, N-sulfonamido, trihalomethane-sulfonamido, halo, carbonyl, cyano, nitro, halo, or amino; and and wherein when R₁ is an indole, R₂ is a hydroxyl, and R₃ is an isopropyl, R₄ is not an adamantane; and pharmaceutically acceptable salts thereof. In a specific embodiment, R₄ is a biphenyl. In another specific embodiment, R₂ is a hydroxyl group, and both racemates are contemplated. In another specific embodiment, R₁ is an indole, and the nitrogen on the indole has a tosyl substituent group.

[0012] A specific embodiment of the invention is a compound of the formula:

wherein R₁ and R₂ are a hydrogen, halo, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, carbonyl, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl; and wherein R₁ and R₂ may be the same or different and are not both hydrogen; and pharmaceutically acceptable salts thereof.

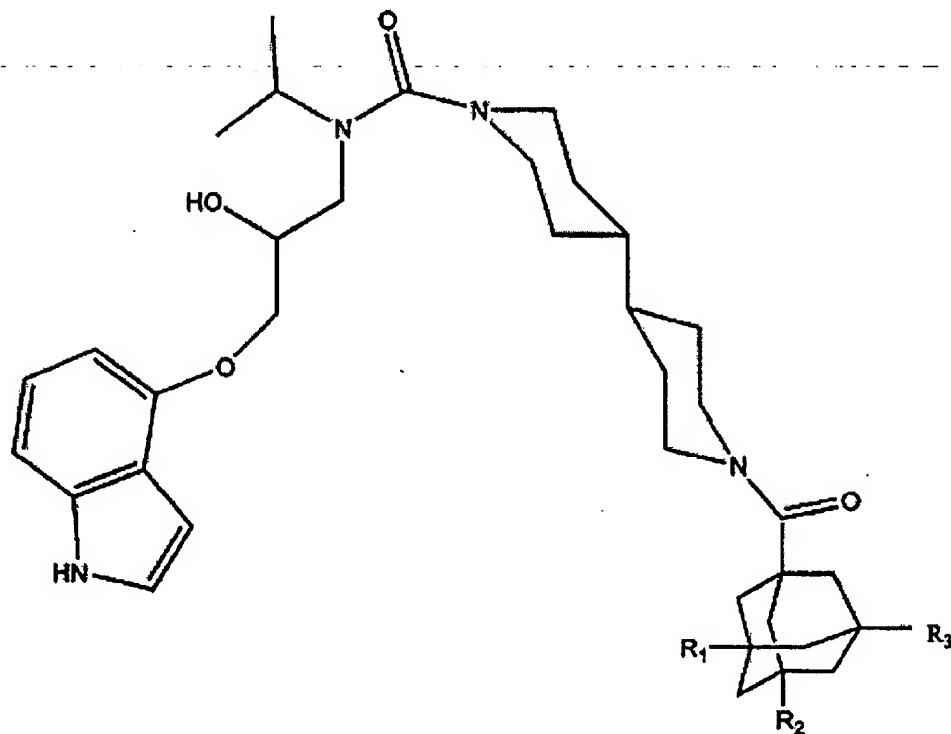


[0013] Another specific embodiment of the invention is a compound of the formula:



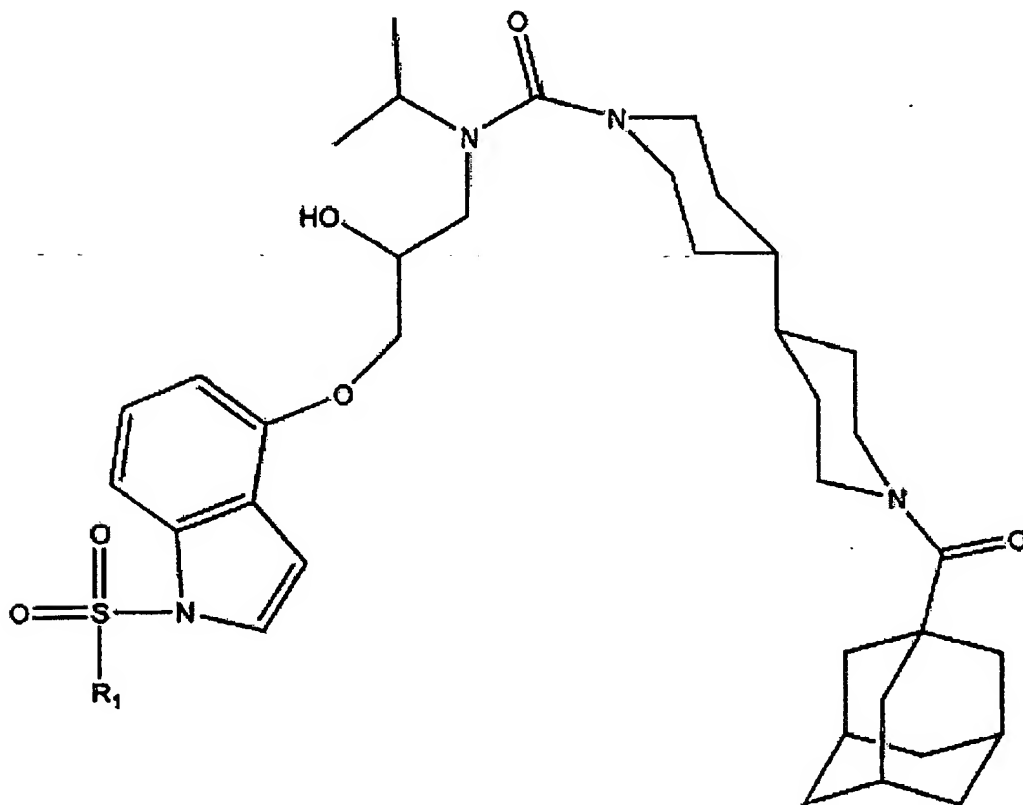
wherein the R_1 is selected from the group of β blockers consisting of propanolol, indenolol, carpindolol, bupranolol, and mepindolol; and pharmaceutically acceptable salts thereof. In a further specific embodiment of the invention, R_1 is propanolol.

[0014] Another specific embodiment of the invention is a compound of the formula:



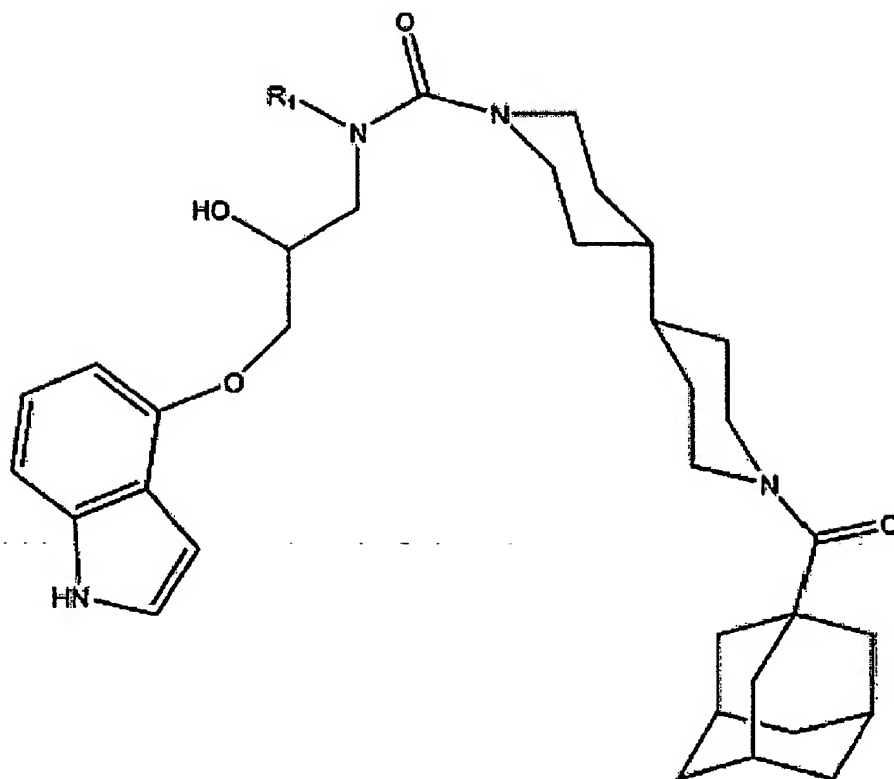
wherein R_1 , R_2 , and R_3 are a hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, carbonyl, halo, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl; and wherein R_1 , R_2 , and R_3 may be the same or at least one is different and are not all hydrogen; and pharmaceutically acceptable salts thereof.

[0015] A further embodiment of the invention is a compound of the formula:



wherein R_1 is a hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, halo, carbonyl, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl. In further specific embodiments of the invention, R_1 is a methyl group, a benzene group, a phenyl, a toluene, a biphenyl, or a naphthalate.

[0016] A compound of the formula:



is also contemplated, wherein R_1 is an alkyl, aminoalkyl, guanidinoalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, halo, carbonyl, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl; and R_1 is not an isopropyl; and pharmaceutically acceptable salts thereof. In further specific embodiments of the invention, R_1 is hydrocarbon chain with 2, 3, 4, or 5 carbons. In further specific embodiments, the hydrocarbon chain may terminate in a hydroxyl group or an amine group.

[0017] An embodiment of the invention is a method of treating breast cancer in a mammal wherein a clinically effective amount of a small molecule inhibitor of Her2 expression is administered to said mammal. A specific embodiment of the invention is a method of treating breast cancer in a mammal, wherein a clinically effective amount of adamanolol, a functionalized adamanolol derivative, or a pharmaceutically acceptable salt thereof is administered to said mammal.

[0018] An embodiment of the invention is a pharmaceutical composition comprising the compound of sentence 1 or pharmaceutically acceptable salts thereof.

[0019] The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described hereinafter which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that the conception and specific embodiment disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims. The novel features which are believed to be characteristic of the invention, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] For a more complete understanding of the present invention, reference is now made to the following descriptions taken in conjunction with the accompanying drawing, in which:

[0021] FIG. 1 demonstrates the interaction between Sur-2 and ESX and its role in regulating Her-2 expression.

[0022] FIG. 2 shows the purification of Sur-2. FIG. 2A shows an SDS-PAGE gel of the purified Sur-2. FIG. 2B shows that *in vitro* translated Sur-2 binds the ESX activation domain, but not its activation-deficient mutant. FIG. 2C shows Sur-2 binds specifically to the ESX activation domain.

[0023] FIG 3 demonstrates the identification of a small Sur-2 domain that binds the ESX activation domain. FIG 3A demonstrates that a deletion mutant of Sur-2 maintained the ability to bind the ESX activation domain. FIG. 3B shows that a series of Sur-2 protein fragments were assayed for the ability to bind the GST fusion of the ESX activation domain (GST-ESX₁₂₉₋₁₅₉). The amino acids 352–625 are sufficient for the interaction with the ESX activation domain. FIG 3C illustrates a model in which overexpression of the small Sur-2 domain specifically inhibits transcriptional activation by the ESX activation domain.

[0024] FIG. 4 indicates the effects of TAT- ESX₁₂₉₋₁₄₅ on the protein levels of Her2 in Her2-expressing breast cancer lines. TAT- ESX₁₂₉₋₁₄₅ significantly reduced the amount of Her2 expression in all three breast cancer lines tested.

[0025] FIG. 5 shows the effects of TAT- ESX₁₂₉₋₁₄₅ on the growth of breast cancer cells. FIG. 5A depicts results of incubating four breast cancer cell lines with TAT- ESX₁₂₉₋₁₄₅. FIG. 5B shows the nuclear condensation induced by TAT- ESX₁₂₉₋₁₄₅. FIG. 5C indicates Her2 overexpression in the three breast cancer lines tested. FIG. 5D indicates that MCF-7 Her18, a Her2 overexpressing cell line, was resistant to ESX₁₂₉₋₁₄₅.

[0026] FIG. 6 shows NMR data for adamanolol and ESX₁₂₉₋₁₄₅. FIG. 6A shows the expansion of the NH- α H region of a NOESY spectrum of ESX₁₂₉₋₁₄₅. FIG. 6B shows the expansion of the NH-NH region of a NOESY spectrum of ESX₁₂₉₋₁₄₅. FIG. 6C shows the summary of inter-residue NOE data as characteristic of an α -helical structure. FIG. 6D shows the results of the NMR studies on adamanolol.

[0027] FIG. 7 show the eight amino-acid segment of ESX that interacts with Sur-2. FIG 7A identifies the minimal ESX peptide that binds Sur-2 *in vitro*. FIG 7B. is a summary of mutational studies. Point mutants of ESX₁₂₉₋₁₄₅ were tested for their ability to bind Sur-2. The indole structure of Trp138 was substituted with Ala, Phe, or Leu, which abolished the interaction

with Sur-2. FIG 7C shows the projection of the minimal binding peptide, ESX₁₃₇₋₁₄₄, onto a helical wheel.

[0028] FIG. 8 shows the activity of adamanolol (7QH2). FIG. 8A shows that adamanolol selectively inhibits the ESX activation domain. FIG. 8B shows that adamanolol selectively impairs the cell growth and viability of Her2-expressing breast cancer cells. FIG. 8C is a western blot showing the inhibition of Her2 by adamanolol. FIG. 8D shows that adamanolol inhibits the ESX-Sur-2 interaction *in vitro*. FIG. 8E shows the estimation of the dissociation constant of the Sur-2 and ESX interaction.

[0029] FIG. 9 shows a screening technique to identify compounds assayed for the ability to inhibit the activity of GAL4-ESX.

[0030] FIG. 10 shows stereoisomers of adamanolol. Adamanolol has two conformers (E and Z) due to the limited rotation of the urea group.

[0031] FIG. 11 shows the synthetic protocol for adamanolol and N-tosyl adamanolol.

[0032] FIG. 12 shows chemical structures of representative pindolol analogs.

[0033] FIG. 13 is a representation of side groups substituents in adamanolol.

[0034] FIG. 14 indicates synthesized functionalized adamanolol derivatives, and their activity (expressed as the IC₅₀) after *in vitro* testing in Her2-expressing breast cancer cell lines. Five compounds are as active as adamanolol, while 2 are more active, and three are about half as active.

[0035] FIG. 15 shows the results of *in vivo* studies of the effect of adamanolol (7QH2) on tumor size in mice.

DETAILED DESCRIPTION OF THE INVENTION

[0036] It is readily apparent to one skilled in the art that various embodiments and modifications can be made to the invention disclosed in this Application without departing from the scope and spirit of the invention.

[0037] As used herein, the use of the word “a” or “an” when used in conjunction with the term “comprising” in the sentences and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

[0038] As used herein, “DRIP-130” is interchangeable with “Sur-2”. “Sur-2” is a Ras-linked subunit of the human mediator complex and comprises SEQ ID NO: 2.

[0039] As used herein “inhibition of Her2 expression” refers to inhibition of the interaction of Sur-2 with ESX, which regulates *Her2* expression. A consequence of Sur-2 inhibition is the decreased transcription of *Her2*. Thus, Sur2 inhibition results in inhibition of Her2 expression.

[0040] As used herein, a “mammal” is an appropriate subject for the method of the present invention. A mammal may be any member of the higher vertebrate class Mammalia, including humans; characterized by live birth, body hair, and mammary glands in the female that secrete milk for feeding the young. Additionally, mammals are characterized by their ability to maintain a constant body temperature despite changing-climatic conditions. Examples of mammals are humans, cats, dogs, cows, mice, rats, and chimpanzees. Mammals may be referred to as “patients”.

[0041] “Peptide” refers to a polymer in which the monomers are amino acids and are joined together through amide bonds, alternatively referred to as a polypeptide. When the amino acids are .alpha.-amino acids, either the L-optical isomer or the D-optical isomer can be used. Additionally, unnatural amino acids, for example, beta-alanine, phenylglycine and homoarginine are also included. The amino acids may be either the D- or L -isomer. The L -isomers are generally preferred. For a general review, see, Spatola, A. F., in CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES AND PROTEINS, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983).

[0042] As used herein, a “peptide mimetic” is a compound that biologically mimics determinants on hormones, cytokines, enzyme substrates, viruses or other bio-molecules, and may antagonize, stimulate, or otherwise modulate the physiological activity of the natural ligands. Certain mimetics that mimic elements of protein secondary and tertiary structure are described in Johnson et al. (1993). The underlying rationale behind the use of peptide mimetics

is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and/or antigen. A peptide mimetic is thus designed to permit molecular interactions similar to the natural molecule.

Molecules are designed to recognize amino acid residues in alpha-helix or beta-turn conformations on the surface of a protein. Such molecules may be used in disrupting certain protein-protein interactions involved in disease. In the present invention, novel compounds are described that inhibit Sur-2. These compounds are alpha-helix mimetics of Sur-2. In a specific embodiment, the inhibitors mimic an eight amino acid (SEQ ID NO: 1) alpha-helical domain of ESX that interacts with Sur-2. One with skill in the art realizes that other alpha-helical domains of ESX may be appropriate targets for small molecule peptide mimetics.

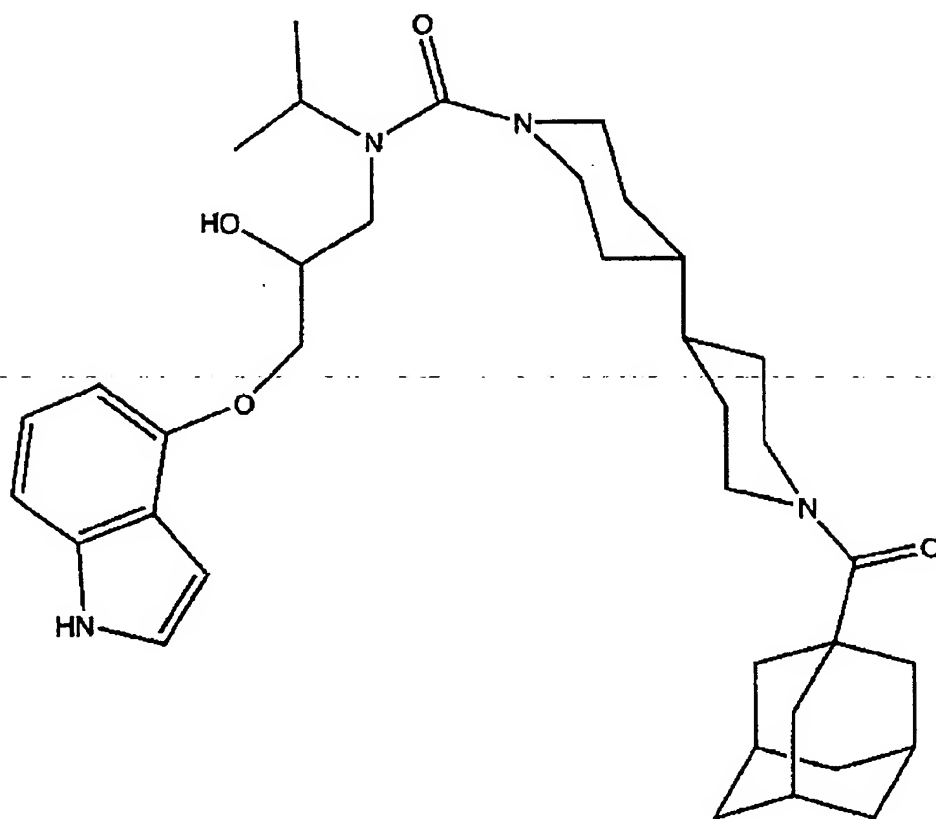
[0043] "Protein", as used herein, means any protein, including, but not limited to peptides, enzymes, glycoproteins, hormones, receptors, antigens, antibodies, growth factors, etc., without limitation. Presently preferred proteins include those comprised of at least 25 amino acid residues, more preferably at least 35 amino acid residues and still more preferably at least 50 amino acid residues.

[0044] Any compounds of this invention containing one or more asymmetric carbon atoms may occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. All such isomeric forms of these compounds are expressly included in the present invention. Each stereogenic carbon may be in the R or S configuration, or a combination of configurations. Some of the compounds of the invention can exist in more than one tautomeric form. The invention includes all such tautomers. The compounds of the invention are only those which are contemplated to be chemically stable as will be appreciated by those skilled in the art. For example, a compound which would have a dangling valency, or a carbanion are not compounds contemplated by the invention. Any of the aromatic ring systems, carbocyclic or heterocyclic, shall be understood to include the non-aromatic ring systems which may be mono- or polyunsaturated, and the positional isomers or analogs thereof. Any of the compounds described herein possessing "nitrogen" and "sulfur" shall include any oxidized form of nitrogen and sulfur and the quaternized form of any basic nitrogen. Unless otherwise defined herein, all nomenclature is as standard in the art.

1. Chemical Definitions and Derivatives

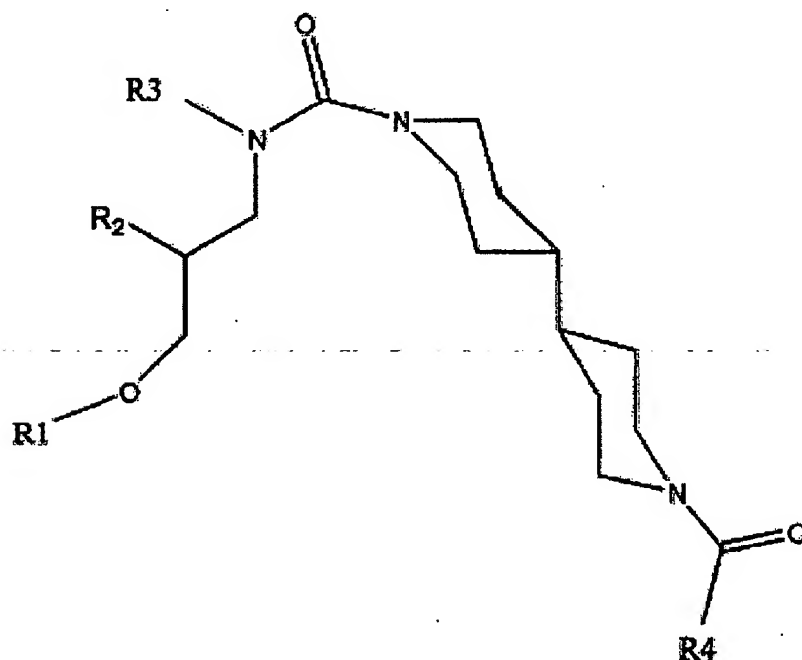
A. Adamanolol

[0045] "Adamanolol" as used herein is defined as a compound consisting of the formula:



[0046] The chemical name for adamanolol is 1'-(adamantane-1-carbonyl)-[4,4'] bipiperidiny-1-carboxylic acid [2-hydroxy-3-3-(1H-indol-4-yloxy)-propyl]-isopropyl-amide.

[0047] "Functionalized adamanolol derivatives" as used herein describe small molecule chemical compound derivatives of adamanolol which retain the Sur-2 inhibition activity of the adamanolol compound, and are generally of the formula:



wherein R_1 is an indole, indole derivative, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, sulfinyl, sulfonyl, carbonyl, cyano, nitro, halo, or amino;

wherein R_2 is a hydrogen, hydroxy, nitrate, halo, loweralkyl, or carbonyl;

wherein R_3 is a halo, loweralkyl, carbonyl, aryl, aralkyl, or heterocycle;

and wherein R_4 is adamantane, adamantane derivative, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy,

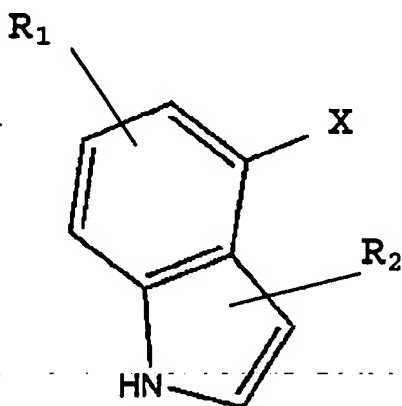
aryloxy, mercapto, alkylthio, arylthio, sulfinyl, sulfonyl, S-sulfonamido, N-sulfonamido, trihalomethane-sulfonamido, halo, carbonyl, cyano, nitro, halo, or amino.

[0048] One with skill in the art realizes that the magnitude of Her2 expression inhibition may vary among functionalized derivatives. In certain embodiments of the invention, the Her2 expression inhibition of the functionalized adamanolol derivatives is less than that of adamanolol, and in other embodiments of the invention the Her2 expression inhibition of the functionalized adamanolol derivatives is greater than that of adamanolol.

B. Indoles

[0049] Indole is a colorless crystalline solid. The heat of combustion at constant volume is 4,268 MJ/mol (1020 kcal/mol). The molecule is planar and has only moderate polarity. Indole has good solubility in a wide range of solvents including petroleum ether, benzene, chloroform, and hot water. Indole forms salts with high concentrations of both strong bases and strong acids. With strong bases, the anion is stabilized by delocalization of the electron over the aromatic system and indole, with a pK for deprotonation of 16.97, is a stronger acid than simple amines. Conversely, because the lone pair of electrons on the nitrogen atom is an integral part of the aromatic system, indole is a much weaker base when combined with strong acids than the alkylamines or pyridine and gives a pK value of -3.5 for protonation. The protonation occurs at the N1 or C3 positions and gives the indolium ion or indoleninium ion.

[0050] Spectroscopic evidence indicates that in strong sulfuric or perchloric acid, protonation occurs at the C3 position. The formation of dimers and trimers complicates the situation and, except for 2-substituted indoles (which, for steric reasons, do not dimerize), an equilibrium exists between the indole, the dimer, the trimer, and their salts. The term "indole derivative" or "substituted indole" as used herein refers to compounds of the general formula:



wherein R₁ and R₂ may be a hydrogen, halo, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, carbonyl, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl, and may be the same or different. In other embodiments of the invention. It is contemplated the ring nitrogen may be substituted, and the substituent group may be a hydrogen, halo, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, carbonyl, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl, and where R₁ and R₂ are not both hydrogen. The term "indole" or "unsubstituted indole" refers the compound of the above formula when R₁ and R₂ are both hydrogen.

[0051] X represents the site of attachment of the indole to the adamanolol or functionalized adamanolol derivative.

C. Beta-blockers

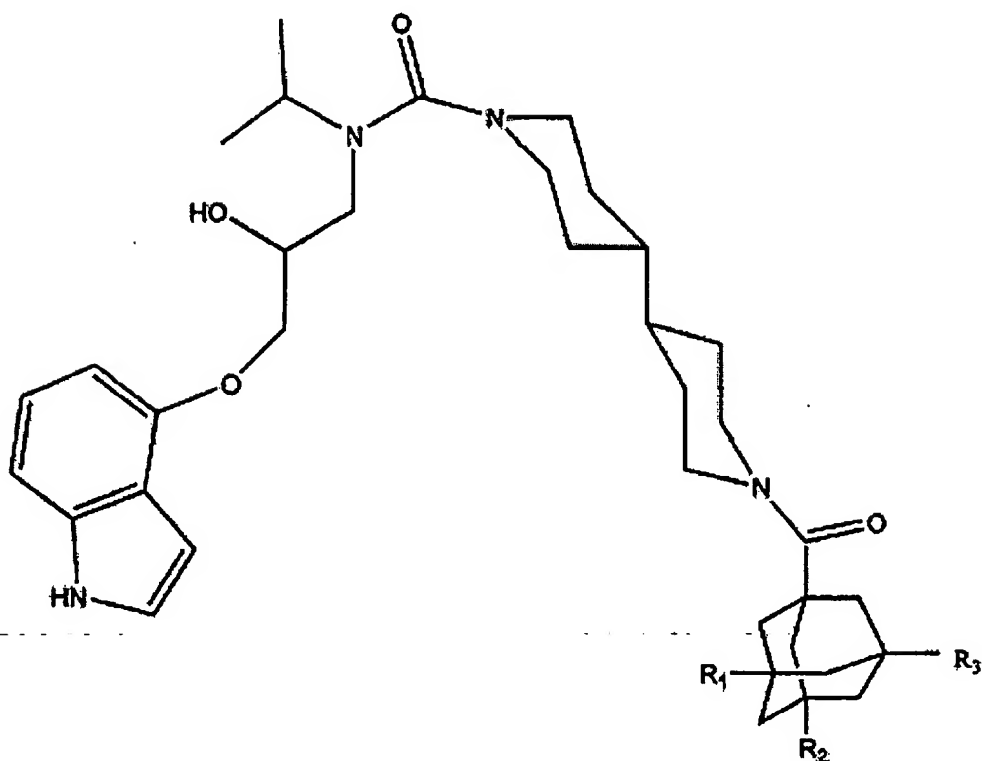
[0052] The beta-blockers comprise a group of drugs that are indicated for use to treat cardiovascular disorders. Nonselective beta-blockers, including propranolol, oxprenolol, pindolol, nadolol, timolol and labetalol, antagonize both β_1 - and β_2 -adrenergic receptors. A common feature in the chemical structure of beta-blockers is that there is at least one aromatic ring structure that is attached to a side alkyl chain possessing a secondary hydroxyl and amine functional group. Each of the available beta-blockers has one or more chiral centers in its structure, and in all cases, at least one of the chiral carbon atoms residing in the alkyl side chain is directly attached to a hydroxyl group. Each of the beta-blockers with one chiral center (e.g., propranolol, metoprolol, atenolol, esmolol, pindolol, and acebutolol), except for timolol, is sold as a racemate.

[0053] One with skill in the art realizes that although the functionized adamanolol derivatives have, in certain embodiments, beta blocker side group substituents, these small molecule derivatives are functionally distinct from beta blockers, and exhibit different pharmacologic activity.

D. Adamantane and derivatives.

[0054] One with skill in the art realizes that adamantane is tricyclo(3,3,1,^{3,7})decane, also described by CAS No.(281-23-2) and EINECS No. (206-001-4). The ten carbon atoms which define the framework structure are arranged in an essentially strainless manner thereby giving a very stable backbone for the addition of a variety of moieties. Four of these carbon atoms, the bridgehead carbons, are tetrahedrally disposed about the center of the molecule. The other six (methylene carbons) are octahedrally disposed. Because of the particular reactivity of adamantane, functional groups have been readily introduced at the bridgehead 1-, 3-, 5-, 7- positions of adamantane. In the present invention, the adamantane group is linked to adamanolol at the 1- position. U.S. Pat. Nos. 5,019,660 to Chapman and Whitehurst and 5,053,434 to Chapman teach diamondoid compounds which bond through the methylene positions of various diamondoid compounds. For a survey of the chemistry of diamondoid molecules, see Adamantane, The Chemistry of Diamond Molecules, Raymond C. Fort, Marcel Dekker, New York, 1976. For synthesis methods for adamantanes, see Paul Schleyer, Cage Hydrocarbons, George A. Olah, ed., Wiley, New York, 1990.

[0055] In specific embodiments, the compound of the formula:



is contemplated.

wherein the adamantane group may be a substituted adamantane derivative such as 1-adamantanol, 1-adamantane carboxylic acid, 1-adamantyl methyl ketone, adamantanone, adamantanol, or 1-bromoadamantane. R₁, R₂, and R₃ may be a hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, carbonyl, halo, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl, and where R₁, R₂, and R₃ may be the same or at least one is different, i.e. all may be different or one may be different. If R₁, R₂, and R₃ are all hydrogen, the group is an “adamantane” or “unsubstituted

adamantane". When R₁, R₂, and R₃ are not all hydrogen, the group is an "adamantane derivative" or "substituted adamantane".

E. Additional side group substituents

[0056] It is contemplated that the functionalized adamanolol derivatives have substituent groups. In specific embodiments, the definitions of those groups are as below.

[0057] The term "alkyl" as used herein refers to a straight, branched, or cyclic hydrocarbon chain fragment or radical containing between about one and about twenty carbon atoms, more preferably between about one and about ten carbon atoms (e.g., methyl, ethyl, n-propyl, iso-propyl, cyclopropyl, n-butyl, iso-butyl, tert-butyl, cyclobutyl, adamantyl, noradamantyl and the like). Straight, branched, or cyclic hydrocarbon chains having eight or fewer carbon atoms will also be referred to herein as "loweralkyl". The hydrocarbon chains may further include one or more degrees of unsaturation, i.e., one or more double or triple bonds (e.g., vinyl, propargyl, allyl, 2-buten-1-yl, 2-cyclopenten-1-yl, 1,3-cyclohexadien-1-yl, 3-cyclohexen-1-yl and the like). Alkyl groups containing double bonds such as just described will also be referred to herein as "alkenes". Similarly, alkyl groups having triple bonds will also be referred to herein as "alkynes". However, as used in context with respect to cyclic alkyl groups, the combinations of double and/or triple bonds do not include those bonding arrangements that render the cyclic hydrocarbon chain aromatic.

[0058] The term "oxo" means a doubly bonded oxygen.

[0059] In addition, the term "alkyl" as used herein further includes one or more substitutions at one or more carbon atoms of the hydrocarbon fragment or radical. Such substitutions include, but are not limited to: aryl; heterocycle; halogen (to form, e.g., trifluoromethyl, --CF₃); nitro (--NO₂); cyano (--CN); hydroxyl (also referred to herein as "hydroxy"), alkoxyl (also referred to herein as alkoxy) or aryloxy (also referred to herein as "aryloxy", --OR); thio or mercapto, alkyl, or arylthio (--SR); amino, alkylamino, arylamino, dialkyl- or diarylamino, or arylalkylamino (--NRR'); aminocarbonyl, alkylaminocarbonyl, arylaminocarbonyl, dialkylaminocarbonyl, diarylamino carbonyl or arylalkylaminocarbonyl (--C(O)NRR'); carboxyl, or alkyl- or aryloxycarbonyl (--C(O)OR); carboxaldehyde, or aryl- or alkylcarbonyl (--C(O)R); iminyl, aryl- or alkyliminyl; sulfo (--SO₂ OR); alkyl- or arylsulfonyl (--SO₂R); carbamido; orthiocarbamido; where R and R' independently are hydrogen, aryl or alkyl

as defined herein. Substituents including heterocyclic groups (i.e., heterocycle, heteroaryl, and heteroaralkyl) are defined by analogy to the above-described terms. For example, the term "heterocycleoxy" refers to the group --OR, where R is heterocycle as defined below.

[0060] The term "methylene" refers to the group --CH₂ --.

[0061] The term "methine" refers to a methylene group for which one hydrogen atom has been replaced by a substituent as described above. The term "methine" can also refer to a methylene group for which one hydrogen atom is replaced by bond to form a hybridized carbon center.

[0062] The term "halo" or "halogen" as used herein refers to the substituents fluoro, bromo, chloro, and iodo.

[0063] The term "carbonyl" as used herein refers to the functional group --C(O)--. However, it will be appreciated that this group may be replaced with well-known groups that have similar electronic and/or steric character, such as thiocarbonyl (--C(S)--); sulfinyl (--S(O)--); sulfonyl (--SO₂ --)phosphonyl (--PO₂ --), and methine. Other carbonyl equivalents will be familiar to those having skill in the medicinal and organic chemical arts.

[0064] The term "aryl" as used herein refers to cyclic aromatic hydrocarbon chains having twenty or fewer carbon atoms, e.g., phenyl, naphthyl, biphenyl and anthryl. One or more carbon atoms of the aryl group may also be substituted with, e.g.: alkyl; aryl; heterocycle; halogen; nitro; cyano; hydroxyl, alkoxy or aryloxy; thio or mercapto, alkyl-, or arylthio; amino, alkylamino, arylamino, dialkyl-, diaryl-, or arylalkylamino; aminocarbonyl, alkylaminocarbonyl, arylaminocarbonyl, dialkylaminocarbonyl, diarylaminocarbonyl or arylalkylaminocarbonyl; carboxyl, or alkyl- or aryloxy carbonyl; carboxaldehyde, or aryl- or alkyl carbonyl; iminyl, or aryl- or alkyliminyl; sulfo; alkyl- or arylsulfonyl; hydroximiny, or aryl- or alkoximinyl; carbamido; or thiocarbamido. In addition, two or more alkyl or heteroalkyl substituents of an aryl group may be combined to form fused aryl-alkyl or aryl-heteroalkyl ring systems (e.g., tetrahydronaphthyl). Substituents including heterocyclic groups (e.g., heterocycleoxy, heteroaryloxy, and heteroaralkylthio) are defined by analogy to the above-described terms.

[0065] The term "aralkyl" as used herein refers to an aryl group that is joined to a parent structure by an alkyl group as described above, e.g., benzyl, .alpha.-methylbenzyl, phenethyl, and the like.

[0066] The term "heterocycle" as used herein refers to a cyclic alkyl group or aryl group as defined above in which one or more carbon atoms have been replaced by a non-carbon atom, especially nitrogen, oxygen, or sulfur. Non-aromatic heterocycles will also be referred to herein as "cyclic heteroalkyl". Aromatic heterocycles are also referred to herein as "heteroaryl". For example, such groups include furyl, tetrahydrofuryl, pyrrolyl, pyrrolidinyl, thienyl, tetrahydrothienyl, oxazolyl, isoxazolyl, triazolyl, thiazolyl, isothiazolyl, pyrazolyl, pyrazolidinyl, oxadiazolyl, thiadiazolyl, imidazolyl, imidazoliny, pyridyl, pyridazinyl, triazinyl, piperidinyl, morpholinyl, thiomorpholinyl, pyrazinyl, piperazinyl, pyrimidinyl, naphthyridinyl, benzofuranyl, benzothienyl, indolyl, indolinyl, indoliziny, indazolyl, quinoliziny, quinoliny, isoquinoliny, cinnoliny, phthalazinyl, quinazoliny, quinoxaliny, pteridinyl, quinuclidiny, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, purinyl, benzimidazolyl, benzthiazolyl, and benzoxazolyl.

[0067] The above heterocyclic groups may further include one or more substituents at one or more carbon and/or non-carbon atoms of the heteroaryl group, e.g.: alkyl; aryl; heterocycle; halogen; nitro; cyano; hydroxyl, alkoxyl or aryloxy; thio or mercapto, alkyl- or arylthio; amino, alkyl-, aryl-, dialkyl- diaryl-, or arylalkylamino; aminocarbonyl, alkylaminocarbonyl, arylaminocarbonyl, dialkylaminocarbonyl, diarylaminocarbonyl or arylalkylaminocarbonyl; carboxyl, or alkyl- or aryloxy carbonyl; carboxaldehyde, or aryl- or alkyl carbonyl; iminyl, or aryl- or alkyliminyl; sulfo; alkyl- or arylsulfonyl; hydroximinyl, or aryl- or alkoximinyl; carbamido; or thiocarbamido. In addition, two or more alkyl substituents may be combined to form fused heterocycle-alkyl or heterocycle-aryl ring systems. Substituents including heterocyclic groups (e.g., heterocycleoxy, heteroaryloxy, and heteroaralkylthio) are defined by analogy to the above-described terms.

[0068] The term "heterocyclealkyl" refers to a heterocycle group that is joined to a parent structure by one or more alkyl groups as described above, e.g., 2-piperidylmethyl, and the like. The term "heteroaralkyl" as used herein refers to a heteroaryl group that is joined to a parent structure by one or more alkyl groups as described above, e.g., 2-thienylmethyl, and the like.

[0069] The term "tosyl" or "Ts" refers to p-toluenesulfonate, shown below. It is the conjugate base of the strong acid, p-toluenesulfonic acid. Sulfonate esters are compounds having the sulfonate group, $-O-S(=O)(=O)$, wherein the single bonded oxygen is bonded directly to carbon, which carbon may be single bonded to any atom but may be multiple bonded only to carbon. The term "mesyl" or "Ms" refers to a mesyl group, which is a sulfonate ester having the formula CH_3SO_2- .

2. Salts and Esters for Pharmaceutical use

[0070] Non-toxic esters and salts which are generally prepared by reacting the free base with a suitable organic or inorganic acid are suitable for pharmaceutical use. Representative salts and esters include the following: Acetate, Lactobionate, Benzenesulfonate, Laurate, Benzoate, Malate, Bicarbonate Maleate, Bisulfate Mandelate, Bitartrate, Mesylate, Borate, Methylbromide, Bromide, Methylnitrate, Calcium Edetate, Methylsulfate, Camsylate, Mucate, Carbonate, Napsylate, Chloride, Nitrate, Clavulanate, N-methylglucamine, Citrate, ammonium salt, Dihydrochloride, Oleate, Edetate, Oxalate, Edisylate, Pamoate (Embonate), Estolate, Palmitate, Esylate, Pantothenate, Fumarate, Phosphate/diphosphate, Gluceptate, Polygalacturonate, Gluconate, Salicylate, Glutamate, Stearate, Glycolylarsanilate, Sulfate, Hexylresorcinolate, Subacetate, Hydrabamine, Succinate, Hydrobromide, Tannate, Hydrochloride, Tartrate, Hydroxynaphthoate, Teoclolate, Iodide, Tosylate, Isothionate, Triethiodide, Lactate, Valerate

3. Pharmaceutical Preparations

[0071] Pharmaceutical compositions of the present invention comprise an effective amount of at least one Sur-2 inhibitors or additional agent dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of an pharmaceutical composition that contains at least one Sur-2 inhibitors or additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (e.g., human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

[0072] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

[0073] The Sur-2 inhibitors (Her2 expression inhibitors) may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. The present invention can be administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, inhalation (e.g., aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in cremes, in lipid compositions (e.g., liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference).

[0074] The actual dosage amount of a composition of the present invention administered to an animal patient can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

[0075] In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, the an active compound may comprise between about 2% to about 75% of the weight of the unit, or between

about 25% to about 60%, for example, and any range derivable therein. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered, based on the numbers described above.

[0076] In any case, the composition may comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (e.g., methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

[0077] The Sur-2 inhibitors may be formulated into a composition in a free base, neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine.

[0078] In embodiments where the composition is in a liquid form, a carrier can be a solvent or dispersion medium comprising but not limited to, water, ethanol, polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol, etc), lipids (e.g., triglycerides, vegetable oils, liposomes) and combinations thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin; by the maintenance of the required particle size by

dispersion in carriers such as, for example liquid polyol or lipids; by the use of surfactants such as, for example hydroxypropylcellulose; or combinations thereof such methods. In many cases, it will be preferable to include isotonic agents, such as, for example, sugars, sodium chloride or combinations thereof.

[0079] In other embodiments, one may use eye drops, nasal solutions or sprays, aerosols or inhalants in the present invention. Such compositions are generally designed to be compatible with the target tissue type. In a non-limiting example, nasal solutions are usually aqueous solutions designed to be administered to the nasal passages in drops or sprays. Nasal solutions are prepared so that they are similar in many respects to nasal secretions, so that normal ciliary action is maintained. Thus, in preferred embodiments the aqueous nasal solutions usually are isotonic or slightly buffered to maintain a pH of about 5.5 to about 6.5. In addition, antimicrobial preservatives, similar to those used in ophthalmic preparations, drugs, or appropriate drug stabilizers, if required, may be included in the formulation. For example, various commercial nasal preparations are known and include drugs such as antibiotics or antihistamines.

[0080] In certain embodiments the Sur-2 inhibitor is prepared for administration by such routes as oral ingestion. In these embodiments, the solid composition may comprise, for example, solutions, suspensions, emulsions, tablets, pills, capsules (e.g., hard or soft shelled gelatin capsules), sustained release formulations, buccal compositions, troches, elixirs, suspensions, syrups, wafers, or combinations thereof. Oral compositions may be incorporated directly with the food of the diet. Preferred carriers for oral administration comprise inert diluents, assimilable edible carriers or combinations thereof. In other aspects of the invention, the oral composition may be prepared as a syrup or elixir. A syrup or elixir, and may comprise, for example, at least one active agent, a sweetening agent, a preservative, a flavoring agent, a dye, a preservative, or combinations thereof.

[0081] In certain preferred embodiments an oral composition may comprise one or more binders, excipients, disintegration agents, lubricants, flavoring agents, and combinations thereof. In certain embodiments, a composition may comprise one or more of the following: a binder, such as, for example, gum tragacanth, acacia, cornstarch, gelatin or combinations thereof; an excipient, such as, for example, dicalcium phosphate, mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate or combinations thereof; a disintegrating agent, such as, for example, corn starch, potato starch, alginic acid or

combinations thereof; a lubricant, such as, for example, magnesium stearate; a sweetening agent, such as, for example, sucrose, lactose, saccharin or combinations thereof; a flavoring agent, such as, for example peppermint, oil of wintergreen, cherry flavoring, orange flavoring, etc.; or combinations thereof the foregoing. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both.

[0082] Additional formulations which are suitable for other modes of administration include suppositories. Suppositories are solid dosage forms of various weights and shapes, usually medicated, for insertion into the rectum, vagina or urethra. After insertion, suppositories soften, melt or dissolve in the cavity fluids. In general, for suppositories, traditional carriers may include, for example, polyalkylene glycols, triglycerides or combinations thereof. In certain embodiments, suppositories may be formed from mixtures containing, for example, the active ingredient in the range of about 0.5% to about 10%, and preferably about 1% to about 2%.

[0083] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The preparation of highly concentrated compositions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small area.

[0084] The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein.

[0085] In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

4. Breast Cancer Treatment

[0086] In the present invention, small molecule inhibitors of Sur-2 are provided which target Her2-expressing breast cancer cells. "Breast cancer" means any of various carcinomas of the breast or mammary tissue. In certain embodiments, it is contemplated that Sur-2 inhibitors will comprise a component of a chemotherapy and radiation treatment regimen in the treatment of breast cancer. Management of the breast cancer currently relies on a combination of early diagnosis through routine breast screening procedures and aggressive treatment. Such treatment may include surgery, radiotherapy, chemotherapy, hormone therapy or combinations of these therapies. Examples of drug treatments currently available for breast cancer include tamoxifen, adriamycin, letrozole, and paclitaxel.

[0087] In other embodiments, it is contemplated that small molecule inhibitors of Sur-2 may be useful in a prophylactic context.

[0088] Overexpression of Her2 has also been found in a portion of ovarian cancers, gastric cancers, endometrial cancers, salivary cancers, pancreatic cancers, prostate cancers, colorectal cancers, and non-small-cell lung cancers. The other cancers associated with overexpression of Her2 are potentially treatable with Sur-2 inhibitors.

[0089] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the present invention, processes, machines, manufacture, compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the present invention.

Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.

EXAMPLES

[0090] The below are nonlimiting examples of the present invention.

Example 1

Materials

[0091] HeLa S3 and MCF7 cells were purchased from American Type Culture Collection. K-BR3, MDA-MB453 and MDA-MB468, were kindly supplied by Dr. Mien-Chie Hung (M. D. Anderson Cancer Center, Houston, TX). MCF7, MCF7-HER18, SK-BR-3, MDA-MB-453, and MDA-MB-468 cells were grown in DMEM/F-12 medium with 10% FBS. Z. Songyang (Baylor College of Medicine) kindly supplied 293Tag cells. The cDNA plasmid construct of Sur-2 (pcDNA3-FLAG-Sur-2) was a kind gift from L. P. Freedman (Memorial Sloan-Kettering Cancer Center). Peptides were synthesized by the fluorenylmethoxycarbonyl chemistry by using Rink amide methylbenzhydrylamine resins, purified by HPLC, and characterized by electrospray ionization mass spectroscopy and/or NMR.

Example 2

Isolation of Sur-2 and interaction with ESX.

[0092] HeLa cell nuclear extracts were prepared as known in the art (Dignam, 1983). GST fusion proteins of the ESX activation domain or its activation-deficient mutant were obtained as described (Choi *et al.*, 2000). The glutathione beads binding GST fusion protein or GST only were incubated with HeLa cell nuclear extracts and then extensively washed by buffer containing 20mM TrisHCl (pH 7.5), 150 mM NaCl, and 0.1% Nonidet P-40. The bound proteins were separated by SDS/PAGE and stained with Coomassie brilliant blue R-250. The protein bands stained with Coomassie brilliant blue R-250 were excised from the gel and microsequenced by mass spectrometry as described. S³⁵-labeled DRIP130 proteins were prepared by TNT T7 Quick Coupled Transcription/translation system (Promega Corporation, Madison, WI). The glutathione beads binding to GST fusion protein or GST only were incubated

with the *in vitro* translated proteins in 500 μ l of binding buffer containing 100 mM NaCl, 0.4% Nonidet P-40, 10% glycerol, 20 mM TrisHCl (pH 7.5), 10 mM MgCl₂, and 2 mM DTT for 1 h at 4°C. After extensive washing with the same buffer, the bound proteins were analyzed by SDS-PAGE.

[0093] Human proteins that bound to the activation domain of ESX from HeLa nuclear extracts (FIG. 2A) were isolated. SDS-PAGE analyses of the isolated proteins showed that a 130-kDa protein bound tightly to the ESX activation domain but not to its activation-deficient mutant protein. Microsequencing data of a tryptic peptide matched to Sur-2 (DRIP130/CRSP130/Sur-2), a metazoan-specific component of the human mediator complexes known as DRIP (vitamin D receptor coactivator complex), CRSP (coactivator complex for Sp1), and ARC (activator-recruited cofactor) complexes. Other proteins that bound to the ESX activation domain (FIG. 2A) were abundant house-keeping proteins such as ATP-citrate lyase and 6-phosphofructokinase, and had affinity, although weaker, even to the activation-deficient mutant as revealed by silver staining of gels. *In vitro* translated Sur-2 bound to the ESX activation domain but not its activation-deficient mutant protein (FIG. 2B). The 17-aa segment of the ESX activation domain (ESX₁₂₉₋₁₄₅) was sufficient for mediating the interaction with Sur-2. Sur-2 seemed to recognize this short segment of ESX in a specific manner because Sur-2 had no detectable affinity to analogous the 17-aa peptides from the activation domains of NF- κ B p65, ALL1, and C/EBP β (FIG. 2C). Sur-2 is a nuclear protein that binds specifically to the activation domain of ESX.

Example 3

Ectopic Expression of a Small Sur-2 Domain Impairs ESX

[0094] To map the minimal domain of Sur-2 that binds the ESX activation domain, a series of 35S-labeled deletion mutant proteins of Sur-2 were incubated with the GST fusion of the ESX activation domain, and the bound proteins were analyzed by SDS/PAGE. The Sur-2 fragment of amino acids 352–625 bound most selectively to the ESX activation domain, and its affinity was as high as that of full-length Sur-2 (FIG. 3A). The domain important for the interaction thus lies in the segment that encompasses residues 352–625. Ectopic expression of this 274-aa fragment or full-length Sur-2 impaired the ability of the ESX activation domain to activate transcription in HEK293T cells, presumably by squelching the ESX activation domain

from binding to endogenous Sur-2-containing mediator complexes (FIG. 3B). In contrast, overexpression of other Sur-2 fragments that had little affinity to the ESX activation domain, Sur-2₁₋₃₅₁ and Sur-2₉₈₃₋₁₂₉₁, had no detectable impacts on the ability of the ESX activation domain to activate transcription. The inhibitory effect is specific to the ESX activation domain because the forced expression of Sur-2 or its fragment had little effect on the activity of the activation domains of NF- κ B p65, ALL1, or C/EBP β . Sur-2 acts, by means of the Sur-2/CRSP/ARC complex, as an ESX-selective coactivator.

Example 4

Sur-2-Binding Peptide Reduces Her2 Expression in Breast Cancer Cells

[0095] Sur-2 is a human homolog of *Clostridium elegans* Sur-2, a protein of unknown function whose mutations suppress the phenotype of an activated *ras* mutation (Singh *et al.*, 1995), and it has been linked to the transcription-activating function of the adenovirus E1A protein and the Elk-1 transcription factor. The functional association of Sur-2 both with the Ras-mediated tumor growth and the Her2 overexpression indicates that the Sur-2-ESX interaction is a target for breast cancer therapy. To test this, we designed a cell-permeable peptide that binds Sur-2 (referred to as TAT-ESX₁₂₉₋₁₄₅). The design takes advantage of the binding affinity of ESX₁₂₉₋₁₄₅ and the cell internalization property of the 11-aa peptide derived from the HIV TAT protein. We first examined whether TAT ESX₁₂₉₋₁₄₅ reduces endogenous expression of the *Her2* gene in three Her2-overexpressing breast cancer cell lines, SK-BR-3, MDA-MB-453, and MCF7 (FIG. 4). Cells were incubated in the presence or absence of TAT-ESX₁₂₉₋₁₄₅, and the protein levels of Her2 were examined by Western blot analyses of normalized amounts of cell lysates. Repeated experiments showed that TAT-ESX₁₂₉₋₁₄₅ reduced the protein levels of Her2 in all three Her2-overexpressing cell lines we tested, whereas the TAT sequence alone or TAT-VP16₄₆₉₋₄₈₅ had no detectable effects. The expression levels of actin were unchanged by TAT-ESX₁₂₉₋₁₄₅, indicating its selective inhibition of Her2 expression.

[0096] The growth of SK-BR-3, MDA-MB-453, and MCF7 cells was completely blocked in the presence of TAT-ESX₁₂₉₋₁₄₅, whereas TAT alone and TAT-VP16₄₆₉₋₄₈₅ had little effects on densation (FIG. 5A). In contrast to the Her2-overexpressing cells, MDA-MB-468, a breast cancer cell line with no detectable levels of Her2, was resistant to TAT-ESX₁₂₉₋₁₄₅ (FIG. 5A and 5B). The limited response of MDA-MB-468 to TAT-ESX₁₂₉₋₁₄₅ demonstrates the specificity

of TAT-ESX₁₂₉₋₁₄₅ in down-regulating the proliferation and viability of Her2-expressing breast cancer cells. The effects of TAT-ESX₁₂₉₋₁₄₅ on an MCF7 cell line stably transfected with the *Her2* gene were examined. The ectopic expression of Her2 on a heterologous promoter effectively rescued MCF7 cells from the TATESX₁₂₉₋₁₄₅-mediated cell death. These results collectively indicate that disruption of the interaction between ESX and DRIP130 decreases Her2 expression and causes cell death in Her2-expressing cancer cells.

Example 5

Characterization of the Interaction of ESX with Sur-2

[0097] ESX₁₂₉₋₁₄₅ exhibited a substantial number of interresidue nuclear Overhauser effects (NOEs) including those between successive amide protons in the main chain and those arising from $d_{\alpha\text{N}}(i, i+3)$ (FIG. 6A and 6B). The pattern of the NOE connectivities is characteristic of the pattern observed for α -helical secondary structures, especially in the region from Ser-137 to Lys-145 (FIG. 6A). Thus, the residues from Ser-137 to Lys-145 have strong helical propensity. Deletion and point mutant proteins of ESX₁₂₉₋₁₄₅ were constructed and examined for their ability to bind Sur-2 *in vitro* (FIG. 7A). The results of the deletion mutants indicate that the residues from Ser-137 to Glu-144 are necessary for the interaction with Sur-2; this 8-aa segment corresponds almost precisely to the region that was detected to be helical by NMR. Substitutions of bulky hydrophobic residues in the short peptide abolished the interaction with Sur-2, whereas mutations of the hydrophilic residues had no detectable effects (FIG. 7B). The projection of residues Ser-137 to Glu-144 onto a helical wheel reveals that these hydrophobic residues all lie along one face of the short helix, perhaps making direct contact with the chemically complementary surface in Sur-2 (FIG. 7C).

Example 6

Identification of adamanolol Sur-2 inhibition activity

[0098] Adamanolol was identified from a chemical library of 2,422 druglike compounds purchased from Tripos Inc. (St. Louis, MO) that contain either indole, benzimidazole, or benzodiazepin in their structures. These library members were carefully selected from the entire compound collection of Tripos, Inc. In addition to the presence of indole-like structures, they have molecular weights suited for inhibitors of protein-protein interactions but carry less than six nitrogen atoms, and their clogP values are between -1 and 6.

[0099] The 2,422 compounds were individually assayed at multiple concentrations for their ability to impair the viability of Her2-overexpressing SK-BR3 cells and Her2-negative MDAMB468 cells. The compounds that selectively impaired the viability and cell growth of SK-BR3 cells were selected and then assayed by transcription reporter gene assay. The compounds that inhibited the ability of the ESX activation domain, but not the VP16 activation domain, to activate the reporter gene expression were selected. Among the selected compounds was adamanolol. (FIG. 3B). The drug-like pindolol derivative impaired the ability of the ESX activation domain to stimulate transcription in cells, whereas it had little effects on that of the VP16 activation domain, a prototypical activator (FIG. 3A). Adamanolol impaired the viability of Her2-positive breast cancer cell lines (SK-BR3, MDA-MB453, and MCF-7), but had much milder effects on MDA-MB468 with no detectable levels of Her2 (FIG. 3B). Western blot analyses of drug-treated cells showed that the expression of Her2 protein, but not that of α -tubulin, was significantly reduced by adamanolol in the Her2-positive SK-BR3 cells.

Example 7

Transcription reporter gene assay

[0100] Mammalian expression vectors each encoding the activation domain of ESX, VP16, or NF κ Bp65 fused with the GAL4 DNA-binding domain were used. Each expression construct was co-transfected to HEK293Tag human kidney cells together with a reporter plasmid in which the production of secreted alkaline phosphatase (SEAP) is under control of an IL-2 promoter carrying five GAL4 binding sites. After a 24 hour incubation in the presence or absence of adamanolol, an aliquot of the culture was assayed for SEAP activity. The total amounts of transfected DNA were normalized.

Example 8

Cell viability assay

[0101] The breast cancer cells were plated on 96-well plates at the density of 5×10^3 cells/well and maintained for 24 hrs. Adamanolol was added to the medium at varied concentrations and incubated for another 24 hrs. Premix WST-1 solution (Takara) was added to each well and incubated for 2 hrs. Cell viability was estimated by measuring absorbance at 450 nm with a SPECTRAmax microplate reader.

Example 9

Effects of adamanolol on the expression levels of Her2 in SK-BR3 cells

[0102] Her2-overexpressing SK-BR3 cells were treated by 7.3 μ M of adamanolol for 24 hrs, and whole cell lysates were analyzed by western blots. The primary antibody used for the western analysis were a monoclonal antibody against Her2 (c-neu-Ab-3; Oncogene Science) and a monoclonal antibody against α -tubulin (T5168; Sigma). The blotted membranes were then incubated with horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin and visualized with ECL chemiluminescence detection reagents (Amersham Bioscience).

Example 10

Fluorescence perturbation study

[0103] To monitor the ESX-DRIP130 interaction, we labeled ESX₁₂₉₋₁₄₅ with fluorescein isothiocyanate (FITC; Pierce) at the Lys₁₄₄ position and measured its fluorescence spectra upon adding the glutathione-S'-transferase (GST) fusion of DRIP130₃₅₂₋₆₂₅. ESX₁₂₉₋₁₄₅ was synthesized by standard Fmoc chemistry (the NH₂ terminus was capped by an acetyl group during the synthesis) and purified by HPLC. The peptide was then coupled with FITC at the Lys₁₄₄ position in a conjugation buffer (100 mM carbonate-bicarbonate buffer, pH 9) in dark and purified by NAP-5 desalting column (Amersham). The labeled peptide was quantified by UV and visible absorbance.

[0104] GST-Sur-2₃₅₂₋₆₂₅ was expressed and purified. Varied concentrations of the fusion protein were added to FITC-ESX₁₂₉₋₁₄₅ (10 nM) in phosphate buffered saline containing 2%(v/v) ethanol, and the fluorescence spectra of FITC-ESX₁₂₉₋₁₄₅ were measured by an ABI LS-50B Fluorescence Spectrometer (Ex: 490 nm). The dissociation constant of the interaction was estimated to be 12 μ M through the emission perturbation at 522 nm.

[0105] To examine the effects of adamanolol on the interaction in vitro, the ethanol component of the binding buffer was replaced with an ethanolic solution of adamanolol. The fractions of FITC-ESX₁₂₉₋₁₄₅ (10 nM) bound to 50 μ M of GST-Sur-2₃₅₂₋₆₂₅ were estimated by measuring fluorescence emission at 522 nm. ESX₁₂₉₋₁₄₅ and pindolol were used as positive and negative controls, respectively (FIG. 3A).

Example 11

NMR studies of adamanolol.

[0106] Adamanolol (0.5 mg) was dissolved in 99.8% chloroform-*d* containing 0.05% (v/v) TMS, and NMR data of the sample were collected by a Bruker AMX600 spectrometer. The assignment of the proton signals was obtained by using a combination of TOCSY, DQF-COSY, and NOESY data sets. In the NOESY spectra, 512 free induction decays were recorded at 298 K with mixing times of 300 and 500 ms. The data were analyzed by the Felix98.0 software (Biosym Technologies) with appropriate apodization and zero-filling to yield real 2D 2K X 2K matrices after reduction. NMR analyses of adamanolol showed signal broadening of the protons around the urea linker at a room temperature and the absence of NOEs between the isopropyl protons and the piperidine even with a 500 msec NOESY mixing time. A reasonable explanation for these NMR characters is a rigid conformation around the urea linker with the preference for the *Z* conformer imposed by the bulky isopropyl group. See FIG. 6.

Example 12

Synthetic scheme for the preparation of adamanolol and functionalized adamanolol derivatives

[0107] The solvents used in reactions were dried prior to use. All other reagents were used as received without purification. All moisture-sensitive reactions were performed in flame-dried and/or oven-dried glassware under a positive pressure of nitrogen unless otherwise noted. Thin-layer chromatography was carried out with glass TLC plates precoated with silica gel 60 F254. Flash column chromatography was accomplished with silica gel BW820MH. Proton nuclear magnetic resonance spectra were recorded in deuterated solvents at 270 or 600 MHz. To a solution of 4-hydroxy indole (1) (0.50 g, 3.8 mmol) in dimethylsulfoxide (10 mL) at 45 ° C was added potassium hydroxide (0.4 g, 7.6 mmol) followed by epichlorohydrin (1.4 g, 15.2 mmol). The reaction mixture was stirred at 45 ° C for 4 h. The reaction was quenched with a saturated solution of NH₄Cl, extracted with diethyl ether (3 × 50 mL). The combined organics were dried with MgSO₄, and concentrated. The product was purified by elution from silica gel column with benzene/acetone mixtures to give the glycidyl 4-indolyl ether (2) (0.75 g, 3.8 mmol, 99%) as a clear oil. To a solution of glycidyl 4-indolyl ether (2) (215 mg, 1.14 mmol) in methanol (2 mL) was added isobutyl amine (160 mg, 2.28 mmol). The solution was heated to 100 ° C and stirred for 32 h. The product was purified by elution from silica gel column with

benzene/acetone mixtures to give the corresponding amine (3) (120 mg, 0.46 mmol, 40%) as a clear oil. To a solution of 4,4'-bipiperidine (420 mg, 5.0 mmol) and triethylamine (2 mL) in 150 mL of CHCl_3 was added a solution of 1-adamantanecarbonyl chloride (4) (500 mg, 2.5 mmol) in 50 mL of CHCl_3 . The resulting mixture was refluxed for 0.5 h, diluted with brine, and dried over MgSO_4 . The solution was then concentrated in vacuum and purified by elution from silica gel column with CHCl_3 /methanol mixtures to give the corresponding amide (5) (750 mg, 2.3 mmol, 90%) as a clear oil. See FIG. 11 and FIG. 13.

Example 13

Synthesis of adamanolol and its isobutyl derivative

[0108] To a solution of amide (120 mg, 0.36 mmol) in 10 mL of THF was added a solution of 1, 1'-carbonyldiimidazole (236 mg, 1.44 mmol) in THF (5 mL). The resulting mixture was refluxed for 4 h. To the reaction mixture was added diethyl ether (20 mL) and washed with brine (3×20 mL). The organic layer was dried with MgSO_4 and concentrated. The carbamoyl imidazole did not require further purification for use in the subsequent steps. Stirring of carbamoyl imidazole with MeI (0.5 mL) in MeCN (2 mL) for 18 h at room temperature. Evaporation of the solvent and excess MeI in vacuum gave the corresponding imidazolium salt quantitatively and again required no additional purification for its conversion to adamanolol. To a solution of imidazolium salt in 2 mL of CH_2Cl_2 was added a solution of pindolol (268 mg, 1.08 mmol) or N-[3-(4-indolyloxy)-2-hydroxypropyl]-isobutyl amine (283 mg, 1.08 mmol) in CH_2Cl_2 (1 mL) and Et_3N (0.5 mL). The resulting mixture was stirred at room temperature for 30 h. Evaporation of the solvent and purified by elution from silica gel column with CHCl_3 /methanol mixtures to gave adamanolol (145 mg, 0.24 mmol, 67%) or its isobutyl derivative (42 mg, 0.07 mmol, 19%) as a clear oil. Adamanolol: ^1H NMR (CDCl_3 , 270 MHz) δ_{H} 7.10 (br, 1H), 7.08-7.04 (m, 2H), 6.58 (d, 1H), 6.51 (d, 1H), 5.33 (br, 1H), 4.52 (br, 2H), 4.39 (m, 1H), 4.37 (br, 1H), 4.15 (br, 2H), 3.69 (d, 1H), 3.43 (m, 1H), 3.30 (br, 1H), 2.65 (br, 4H), 2.02 (s, 3H), 1.98 (s, 6H), 1.71 (s, 6H), 1.34 (dd, 6H), 1.45-0.86 (m, 10H); MS (FAB) ($\text{M} + \text{H}^+$) 605. See FIG. 11.

Example 14

Preparation of N-tosyl adamanolol

[0109] To a solution of adamanolol (8) (30.1 mg, 0.05 mmol) in 1 mL of THF was cooled to 0°C , followed by the addition of 60% sodium hydride in mineral oil (12 mg, 0.50

mmol) and tosyl chloride (10.4 mg, 0.05 mmol). After being stirred at this temperature for 0.5 h, the reaction mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched with brine, extracted with CH_2Cl_2 (3×20 mL). The combined organics were dried with MgSO_4 and concentrated. The product was purified by elution from silica gel column with CHCl_3 /methanol mixtures to give the N-tosyl adamanolol (10) (21.5 mg, 0.03 mmol, 57%) as a clear oil. See FIG. 11.

Example 15

Animal Studies

[0110] Breast cancer cell lines derived from BT474 after one round (BT474M1) or two rounds (BT474M2) of tumorigenic selection were used. 5×10^6 cells were injected with Matrigel into mammary fat pads of nude mice. Two tumors were inoculated for each animal and each treatment group contained 5 animals. Five weeks after inoculation when the tumors were well established (about 100 mm^3 in average) the treatment started (day 0 in the figure). Each mouse received adamanolol (7QH2) at a daily dose of 20 mg/kg through intraperitoneal injection for 5 consecutive days in each week for two weeks. Tumor volumes were measured every three days. The percentile of the tumor volume related to the initial volume at day 0 was plotted for each time point. Results are shown in FIG. 15. Adamanolol is a potent tumor growth inhibitor of HER-2/neu-overexpressing cancer cells *in vivo*. These results show a significant growth suppression of BT474 cells by the treatment.

REFERENCES

[0111] All patents and publications mentioned in the specifications are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

U.S. Patent No. 5,019,660

U.S. Patent No. 5,053,434

- Andreoli, J.M., S.I. Jang, E. Chung, C.M. Coticchia, P.M. Steinert, and N.G. Markova. 1997. The expression of a novel, epithelium-specific ets transcription factor is restricted to the most differentiated layers in the epidermis. *Nucleic Acids Res* 25, no. 21:4287-95.
- Asada, S., Y. Choi, M. Yamada, S.C. Wang, M.C. Hung, J. Qin, and M. Uesugi. 2002. External control of Her2 expression and cancer cell growth by targeting a Ras-linked coactivator. *Proc Natl Acad Sci U S A* 99, 12747-12752.
- Bauer, W., U. Briner, W. Doepfner, R. Haller, R. Huguenin, P. Marbach, T.J. Petcher, and Pless. 1982. SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci* 31, no. 11:1133-40.
- Boyer, T.G., M.E. Martin, E. Lees, R.P. Ricciardi, and A.J. Berk. 1999. Mammalian Srb/Mediator complex is targeted by adenovirus E1A protein [see comments]. *Nature* 399, no. 6733:276-9.
- Bridges, A.J. 1999. The rationale and strategy used to develop a series of highly potent, irreversible, inhibitors of the epidermal growth factor receptor family of tyrosine kinases. *Curr Med Chem* 6, no. 9:825-43.
- Chang, C.H., G.K. Scott, M.A. Baldwin, and C.C. Benz. 1999. Exon 4-encoded acidic domain in the epithelium-restricted Ets factor, ESX, confers potent transactivating capacity and binds to TATA-binding protein (TBP). *Oncogene* 18, no. 25:3682-95.
- Chang, C.H., G.K. Scott, W.L. Kuo, X. Xiong, Y. Suzdaltseva, J.W. Park, P. Sayre, K. Erny, C. Collins, J.W. Gray, and C.C. Benz. 1997. ESX: a structurally unique Ets overexpressed early during human breast tumorigenesis. *Oncogene* 14, no. 13:1617-22.
- Chiang, S.Y., R.W. Burli, C.C. Benz, L. Gawron, G.K. Scott, P.B. Dervan, and T.A. Beerman. 2000. Targeting the ets binding site of the HER2/neu promoter with pyrrole-imidazole polyamides. *J Biol Chem* 275, no. 32:24246-54.
- Choi, Y., S. Asada, and M. Uesugi. 2000. Divergent hTAFII31-binding motifs hidden in activation domains [In Process Citation]. *J Biol Chem* 275, no. 21:15912-6.
- Collins, J.L., P.J. Dambek, S.W. Goldstein, and W.S. Faraci. 1992. CP-99,711: a non-peptide glucagon receptor antagonist. *Bioorg Med Chem Lett* 2:915-918.
- Dignam JD, Martin PL, Shastry BS, Roeder R 1983; Eukaryotic gene transcription with purified components 101:582-98.
- Drebin, J.A., V.C. Link, D.F. Stern, R.A. Weinberg, and M.I. Greene. 1985. Down-modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. *Cell* 41, no. 3:695-706.
- Drebin, J.A., V.C. Link, R.A. Weinberg, and M.I. Greene. 1986. Inhibition of tumor growth by a monoclonal antibody reactive with an oncogene-encoded tumor antigen. *Proc Natl Acad Sci U S A* 83, no. 23:9129-33.

Drews, J. 2000. Drug discovery: a historical perspective [see comments]. *Science* 287, no. 5460:1960-4.

Ebbinghaus, S.W., J.E. Gee, B. Rodu, C.A. Mayfield, G. Sanders, and D.M. Miller. 1993. Triplex formation inhibits HER-2/neu transcription in vitro. *J Clin Invest* 92, no. 5:2433-9.

Ebbinghaus, S.W., H. Fortinberry, and H.B. Gamper, Jr. 1999. Inhibition of transcription elongation in the HER-2/neu coding sequence by triplex-directed covalent modification of the template strand. *Biochemistry* 38, no. 2:619-28.

Foster, B.A., H.A. Coffey, M.J. Morin, and F. Rastinejad. 1999. Pharmacological rescue of mutant p53 conformation and function [see comments]. *Science* 286, no. 5449:2507-10.

Gusterson, B.A., R.D. Gelber, A. Goldhirsch, K.N. Price, J. Save-Soderborgh, R. Anbazhagan, J. Styles, C.M. Rudenstam, R. Golouh, R. Reed, and et al. 1992. Prognostic importance of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. *J Clin Oncol* 10, no. 7:1049-56.

Guy, C.T., M.A. Webster, M. Schaller, T.J. Parsons, R.D. Cardiff, and W.J. Muller. 1992. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc Natl Acad Sci U S A* 89, no. 22:10578-82.

Hassig, C.A., J.K. Tong, and S.L. Schreiber. 1997. Fiber-derived butyrate and the prevention of colon cancer. *Chem Biol* 4, no. 11:783-9.

Taunton, J., C.A. Hassig, and S.L. Schreiber. 1996. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* 272, no. 5260:408-11.

Hollywood, D.P., and H.C. Hurst. 1995. Targeting gene transcription: a new strategy to down-regulate c-erbB-2 expression in mammary carcinoma. *Br J Cancer* 71, no. 4:753-7.

Kwon, H.J., T. Owa, C.A. Hassig, J. Shimada, and S.L. Schreiber. 1998. Depudecin induces morphological reversion of transformed fibroblasts via the inhibition of histone deacetylase. *Proc Natl Acad Sci U S A* 95, no. 7:3356-61.

Lin, R.J., L. Nagy, S. Inoue, W. Shao, W.H. Miller, Jr., and R.M. Evans. 1998. Role of the histone deacetylase complex in acute promyelocytic leukaemia. *Nature* 391, no. 6669:811-4.

Nagahara, H., A.M. Vocero-Akbani, E.L. Snyder, A. Ho, D.G. Latham, N.A. Lissy, M. Becker-Hapak, S.A. Ezhevsky, and S.F. Dowdy. 1998. Transduction of full-length TAT fusion proteins into mammalian cells: TAT-p27Kip1 induces cell migration. *Nat Med* 4, no. 12:1449-52.

Naar, A.M., P.A. Beaurang, S. Zhou, S. Abraham, W. Solomon, and R. Tjian. 1999. Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature* 398, no. 6730:828-32.

Nakanishi T, Oka T, Akagi T. 2001. Recent advances in DNA microarrays. *Acta Med Okayam*, no. 55(6):319-28.

- Noonberg, S.B., G.K. Scott, C.A. Hunt, M.E. Hogan, and C.C. Benz. 1994. Inhibition of transcription factor binding to the HER2 promoter by triplex-forming oligodeoxyribonucleotides. *Gene* 149, no. 1:123-6.
- Pasternak, A., Y. Pan, D. Marino, P.E. Sanderson, R. Mosley, S.P. Rohrer, E.T. Birzin, S.E. Huskey, T. Jacks, K.D. Schleim, K. Cheng, J.M. Schaeffer, A.A. Patchett, and L. Yang. 1999. Potent, orally bioavailable somatostatin agonists: good absorption achieved by urea backbone cyclization. *Bioorg Med Chem Lett* 9, no. 3:491-6.
- Patchett A. 1998. Synthesis and biological activities of potent peptidomimetics selective for somatostatin receptor subtype 2. *Proc Natl Acad Sci U S A* 95, no. 18:10836-41.
- Perou, C.M., T. Sorlie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey, C.A. Rees, J.R. Pollack, D.T. Ross, H. Johnsen, L.A. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, S.X. Zhu, P.E. Lonning, A.L. Borresen-Dale, P.O. Brown, and D. Botstein. 2000. Molecular portraits of human breast tumours. *Nature* 406, no. 6797:747-52.
- Poitout, L., P. Roubert, M.O. Contour-Galcerá, C. Moinet, J. Lannoy, J. Pommier, P. Plas, D. Bigg, and C. Thuriéau. 2001. Identification of potent non-peptide somatostatin antagonists with sst(3) selectivity. *J Med Chem* 44, no. 18:2990-3000.
- Porumb, H., H. Gousset, R. Letellier, V. Salle, D. Briane, J. Vassy, M. Amor-Gueret, L. Israel, and E. Taillandier. 1996. Temporary ex vivo inhibition of the expression of the human oncogene HER2 (NEU) by a triple helix-forming oligonucleotide. *Cancer Res* 56, no. 3:515-22.
- Rachez, C., B.D. Lemon, Z. Suldán, V. Bromleigh, M. Gamble, A.M. Naar, H. Erdjument-Bromage, P. Tempst, and L.P. Freedman. 1999. Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. *Nature* 398, no. 6730:824-8.
- Reichlin, S. 1983. Somatostatin. *N Engl J Med* 309, no. 24:1495-501.
- Roh, H., J.A. Pippin, D.W. Green, C.B. Boswell, C.T. Hirose, N. Mokadam, and J.A. Drebin. 2000. HER2/neu antisense targeting of human breast carcinoma. *Oncogene* 19, no. 53:6138-43.
- Roh, H., J. Pippin, and J.A. Drebin. 2000. Down-regulation of HER2/neu expression induces apoptosis in human cancer cells that overexpress HER2/neu. *Cancer Res* 60, no. 3:560-5.
- Ross, J.S., and J.A. Fletcher. 1999. HER-2/neu (c-erb-B2) gene and protein in breast cancer. *Am J Clin Pathol* 112, no. 1 Suppl 1:S53-67.
- Ryu, S., S. Zhou, A.G. Ladurner, and R. Tjian. 1999. The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. *Nature* 397, no. 6718:446-50.
- Schwarze, S.R., A. Ho, A. Vocero-Akbani, and S.F. Dowdy. 1999. In vivo protein transduction: delivery of a biologically active protein into the mouse [see comments]. *Science* 285, no. 5433:1569-72.

Scott, G.K., J.C. Daniel, X. Xiong, R.A. Maki, D. Kabat, and C.C. Benz. 1994. Binding of an ETS-related protein within the DNase I hypersensitive site of the HER2/neu promoter in human breast cancer cells. *J Biol Chem* 269, no. 31:19848-58.

Singh, N., and M. Han. 1995. sur-2, a novel gene, functions late in the let-60 ras-mediated signaling pathway during *Caenorhabditis elegans* vulval induction. *Genes Dev* 9, no. 18:2251-65.

Slamon, D.J., W. Godolphin, L.A. Jones, J.A. Holt, S.G. Wong, D.E. Keith, W.J. Levin, S.G. Stuart, J. Udove, A. Ullrich, and et al. 1989. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244, no. 4905:707-12.

Slamon, D.J., G.M. Clark, S.G. Wong, W.J. Levin, A. Ullrich, and W.L. McGuire. 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235, no. 4785:177-82.

Stevens, J.L., G.T. Cantin, G. Wang, A. Shevchenko, and A.J. Berk. 2002. Transcription control by E1A and MAP kinase pathway via Sur2 mediator subunit. *Science* 296, no. 5568:755-8.

Takase, Y., T. Saeki, N. Watanabe, H. Adachi, S. Souda, and I. Saito. 1994. Cyclic GMP phosphodiesterase inhibitors. 2. Requirement of 6-substitution of quinazoline derivatives for potent and selective inhibitory activity. *J Med Chem* 37, no. 13:2106-11.

Veber, D.F. 1992. Design and discovery in the development of peptide analogs. In *Peptides; Chemistry and Biology: Proceedings of the 12th American Peptide Symposium*; Smith, J. A., Rivier, J. E., Eds.; ESCOM: Leiden:pp 3-14.

Yang, L., S.C. Berk, S.P. Rohrer, R.T. Mosley, L. Guo, D.J. Underwood, B.H. Arison, E.T. Birzin, E.C. Hayes, S.W. Mitra, R.M. Parmar, K. Cheng, T.J. Wu, B.S. Butler, F. Foor, A. Pasternak, Y. Pan, M. Silva, R.M. Freidinger, R.G. Smith, K. Chapman, J.M. Schaeffer, and Rohrer, S.P., E.T. Birzin, R.T. Mosley, S.C. Berk, S.M. Hutchins, D.M. Shen, Y. Xiong, E.C. Hayes, R.M. Parmar, F. Foor, S.W. Mitra, S.J. Degrado, M. Shu, J.M. Klopp, S.J. Cai, A. Blake, W.W. Chan, A. Pasternak, L. Yang, A.A. Patchett, R.G. Smith, K.T. Chapman, and J.M. Schaeffer. 1998. Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. *Science* 282, no. 5389:737-40.

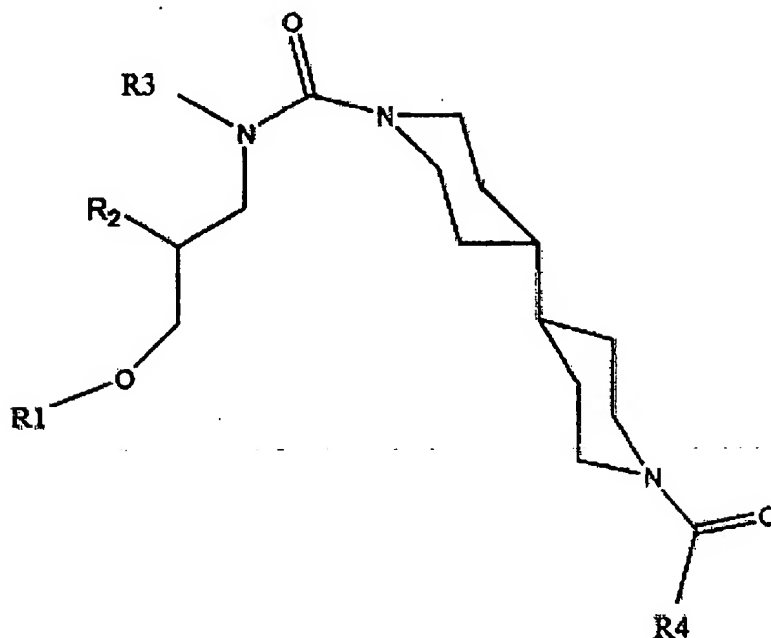
Yu, D., B. Liu, T. Jing, D. Sun, J.E. Price, S.E. Singletary, N. Ibrahim, G.N. Hortobagyi, and M.C. Hung. 1998. Overexpression of both p185c-erbB2 and p170mdr-1 renders breast cancer cells highly resistant to taxol. *Oncogene* 16, no. 16:2087-94.

Yu, D.H., and M.C. Hung. 1991. Expression of activated rat neu oncogene is sufficient to induce experimental metastasis in 3T3 cells. *Oncogene* 6, no. 11:1991-6.

Zhou, B.P., M.C. Hu, S.A. Miller, Z. Yu, W. Xia, S.Y. Lin, and M.C. Hung. 2000. HER-2/neu blocks tumor necrosis factor-induced apoptosis via the Akt/NF-kappaB pathway. *J Biol Chem* 275, no. 11:8027-31.

What is claimed is:

1. A compound of the formula:



wherein R₁ is an indole, indole derivative, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, sulfinyl, sulfonyl, carbonyl, cyano, nitro, halo, or amino;

wherein R₂ is a hydrogen, hydroxy, nitrate, halo, loweralkyl, or carbonyl;

wherein R₃ is a halo, loweralkyl, carbonyl, aryl, aralkyl, or heterocycle;

and wherein R₄ is adamantane, adamantane derivative, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, sulfinyl, sulfonyl, S-sulfonamido, N-

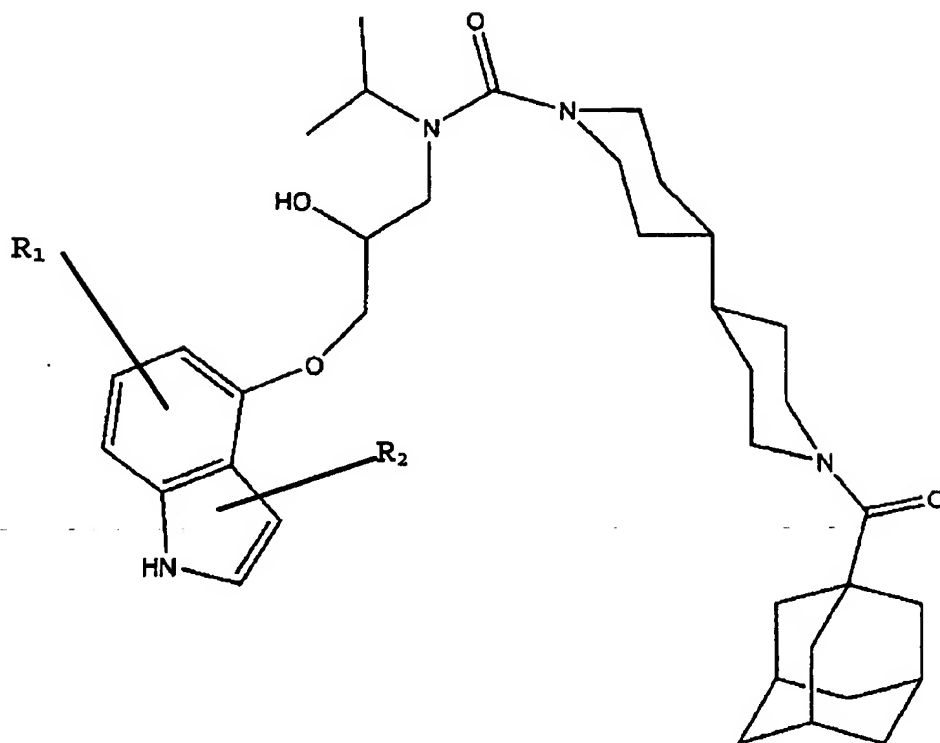
sulfonamido, trihalomethane-sulfonamido, halo, carbonyl, cyano, nitro, halo, or amino;

and wherein when R₁ is an indole, R₂ is a hydroxyl, and R₃ is an isopropyl, R₄ is not an adamantane;

and pharmaceutically acceptable salts thereof.

2. The compound of claim 1, wherein R₁ is a benzene, xylene, cumene, toluene, pentathene, indene, naphthalene, indacene, pyrimidine, indolyl, purine, quinoline, or isoquinoline.
3. The compound of claim 1, wherein R₂ is a methane, ethane, propane, or hydroxyl.

4. A compound of the formula:



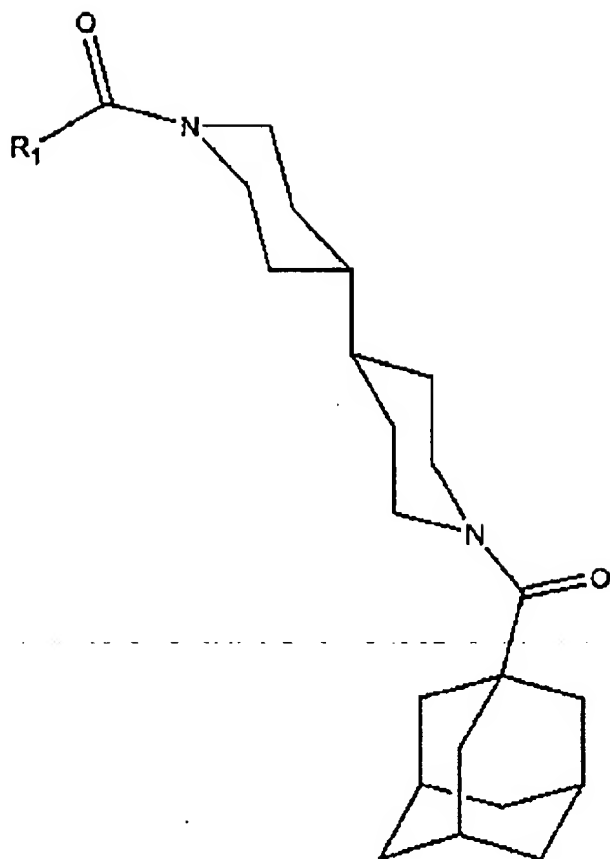
wherein R_1 and R_2 are a hydrogen, halo, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, carbonyl, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl;

and wherein R_1 and R_2 may be the same or different;

and wherein R_1 and R_2 are not both hydrogen;

and pharmaceutically acceptable salts thereof.

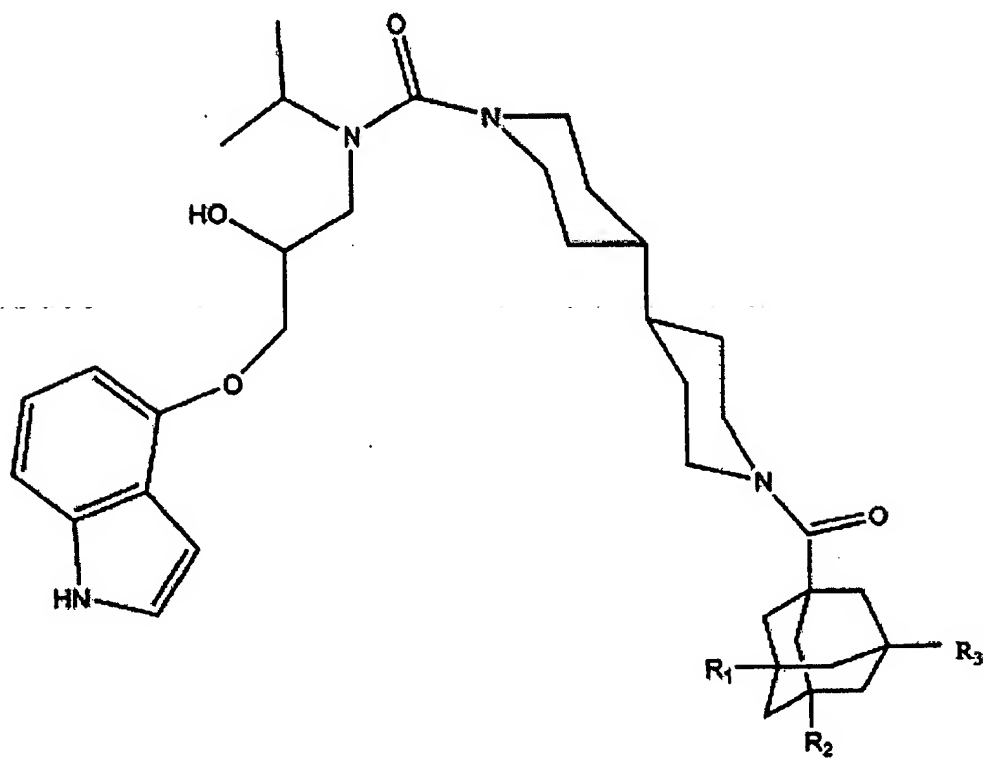
5. A compound of the formula:



wherein the R₁ is selected from the group of β blockers consisting of propanolol, indenolol, carbindolol, bupranolol, and mepindolol;

and pharmaceutically acceptable salts thereof.

6. The compound of claim 5 wherein the β blocker substituent group is propanolol.
7. A compound of the formula:



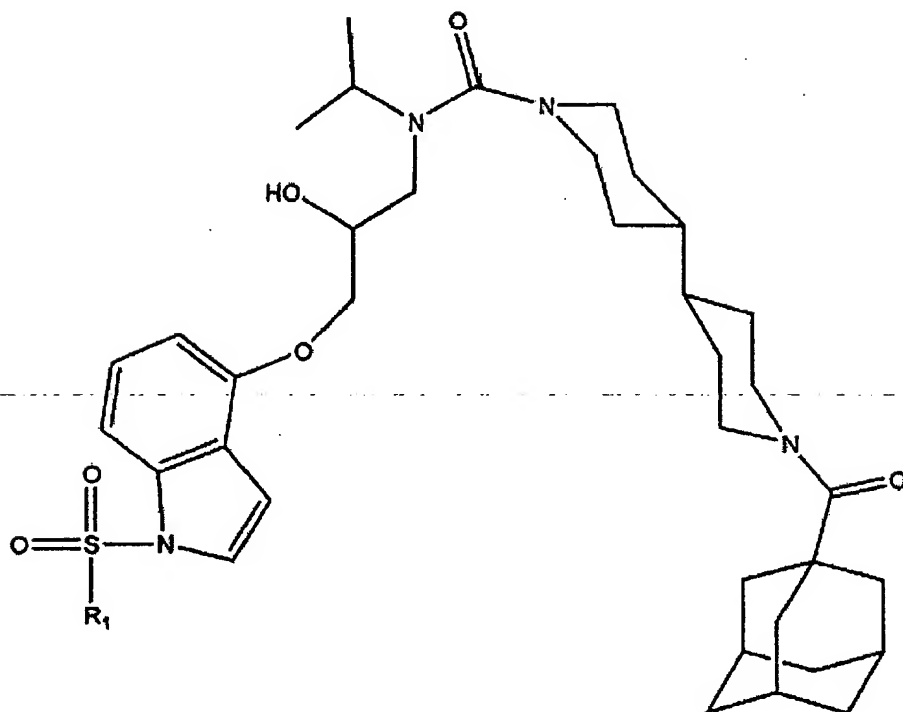
wherein R_1 , R_2 , and R_3 are a hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, carbonyl, halo, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl;

and wherein R_1 , R_2 , and R_3 may be the same or at least one is different;

and wherein R_1 , R_2 , and R_3 are not all hydrogen;

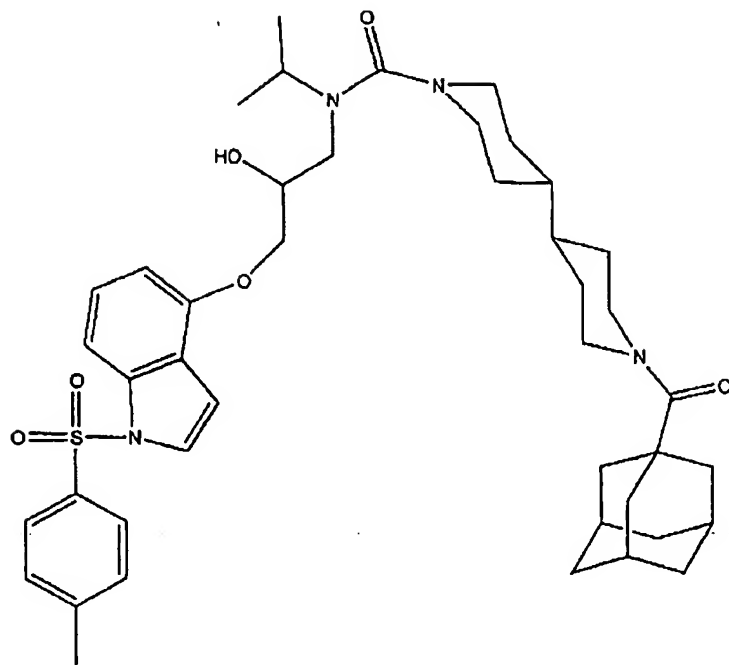
and pharmaceutically acceptable salts thereof.

8. A compound of the formula:

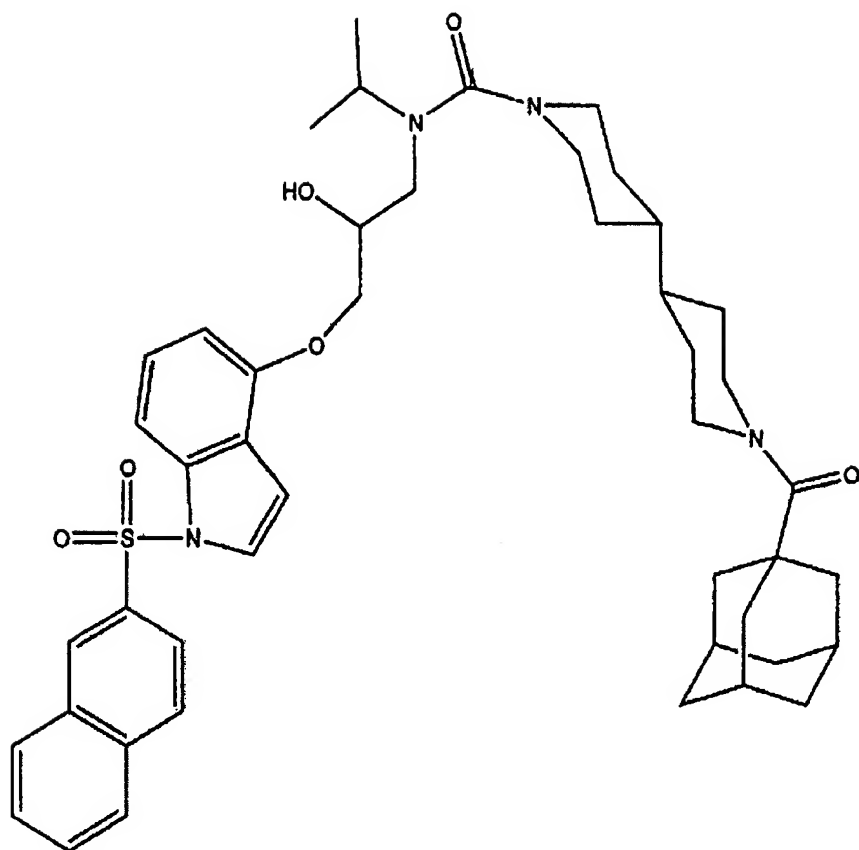


wherein R₁ is a hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, halo, carbonyl, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl.

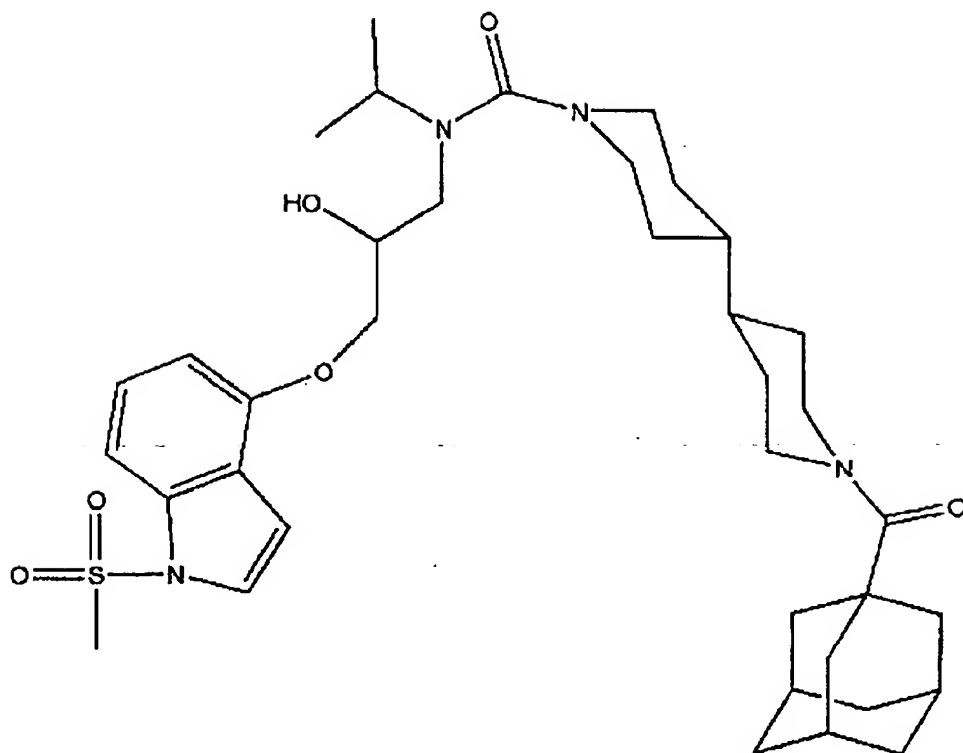
9. The compound of claim 8, selected from the group consisting of:
formula (I)



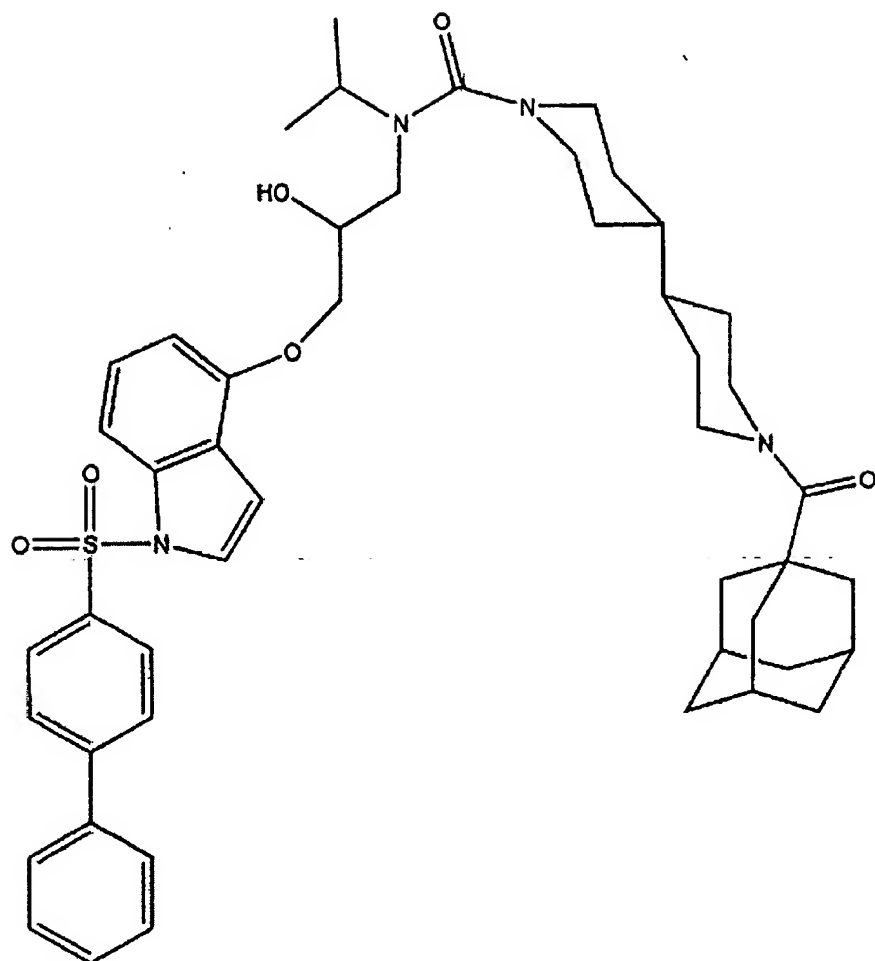
formula (II)



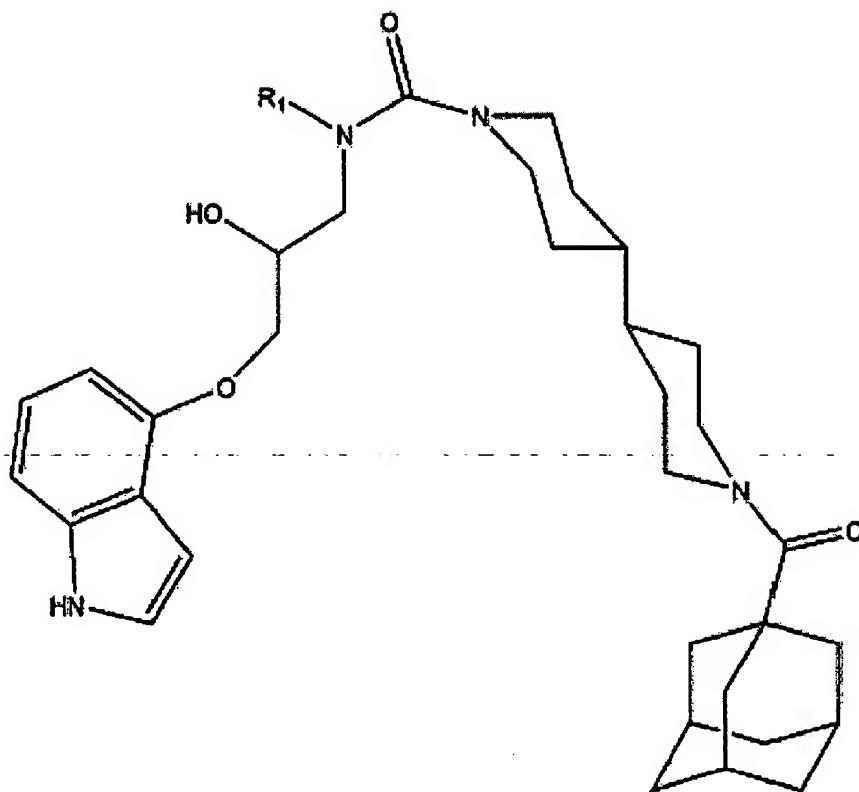
formula (III)



and formula (IV)



10. A compound of the formula:

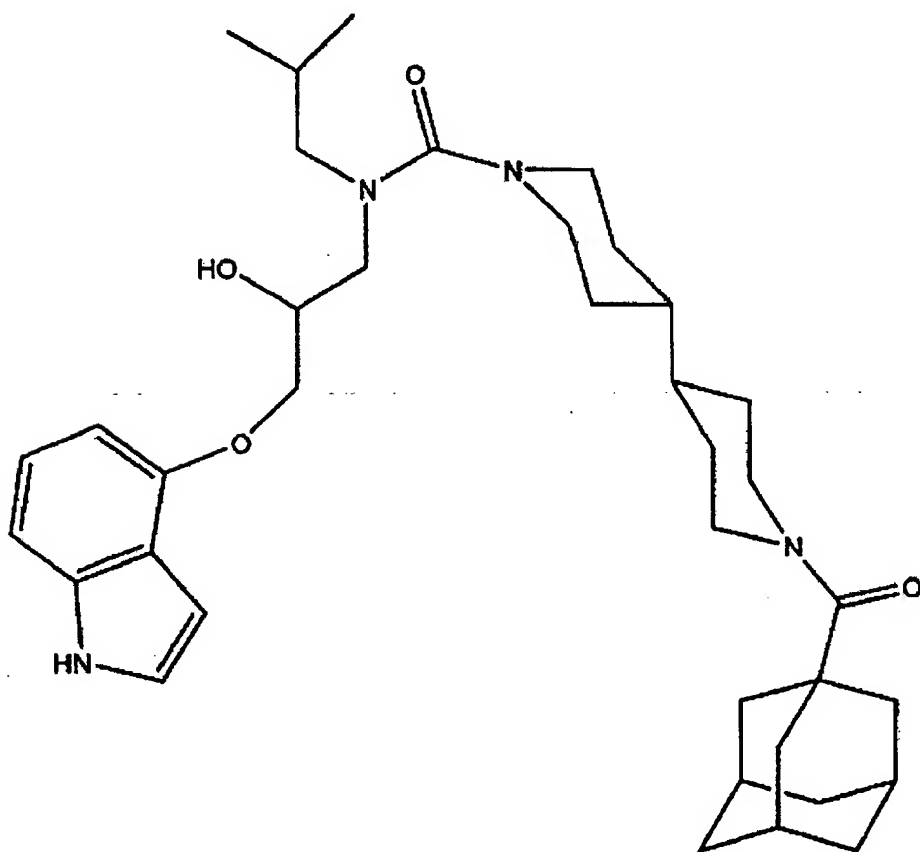


wherein R_1 is an alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, halo, carbonyl, acetyl, carboxy, sulfonyl or trihalomethane-sulfonyl;

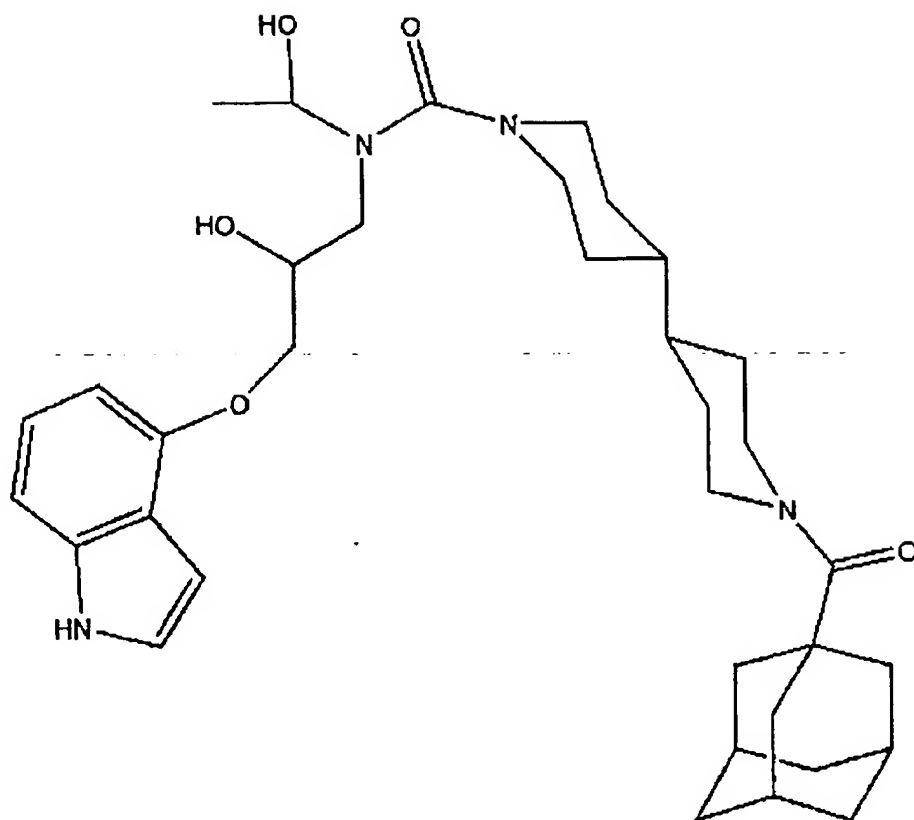
and wherein R_1 is not isopropyl;

and pharmaceutically acceptable salts thereof.

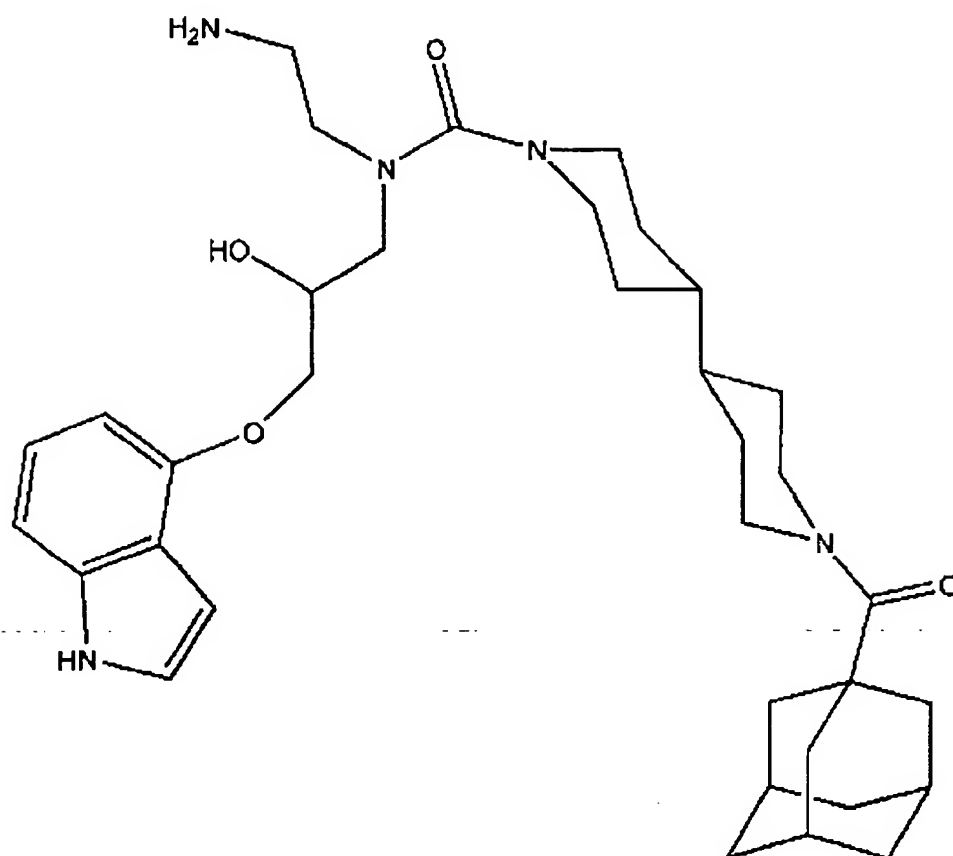
11. The compound of claim 10, wherein R_1 is a methane, ethane, propane, butane, pentane, hexane, heptane, octane, isobutyl, *sec*-butyl, *tert*-butyl, isopentyl, *tert*-pentyl, neo-pentyl, or isohexyl.
12. The compound of claim 10 selected from the group consisting of:
formula (I)



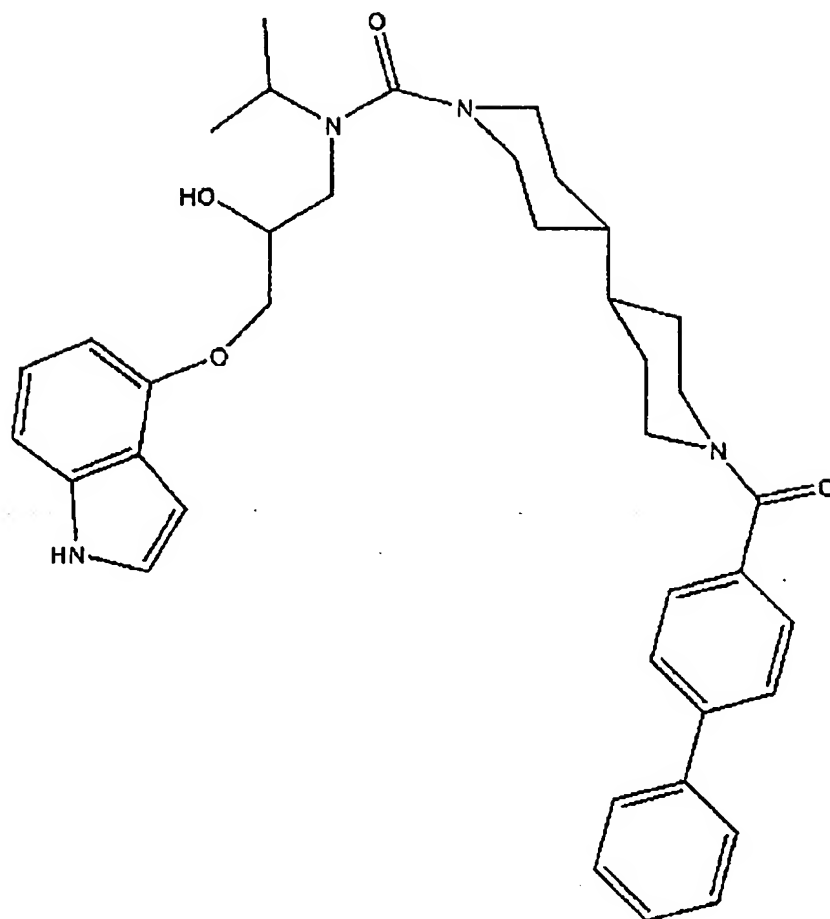
formula (II)



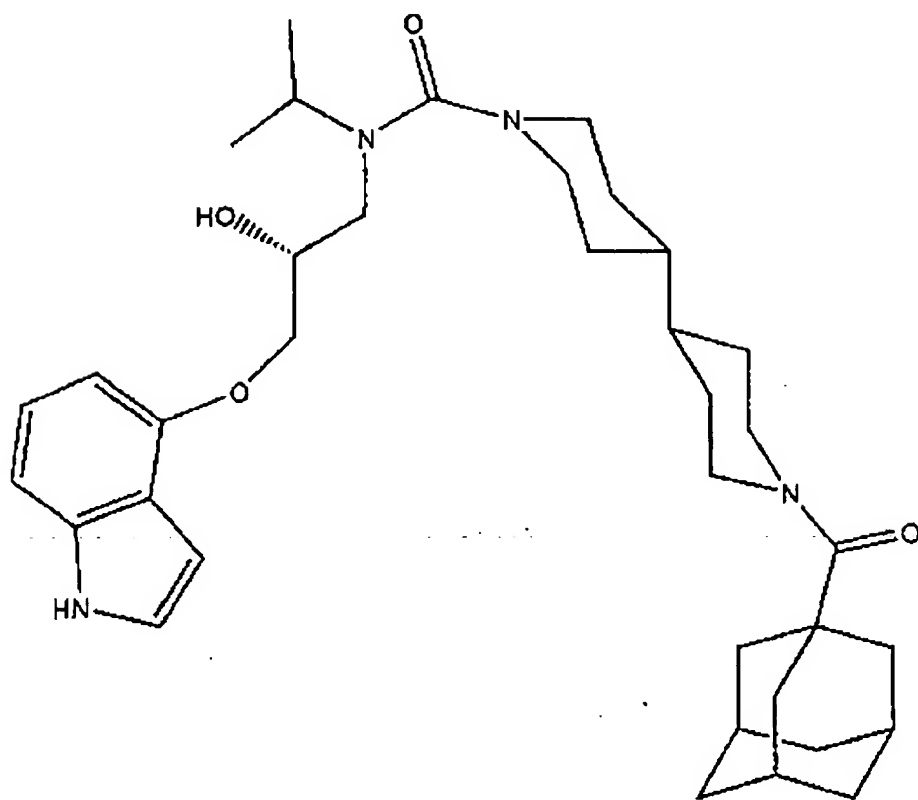
and formula (III)



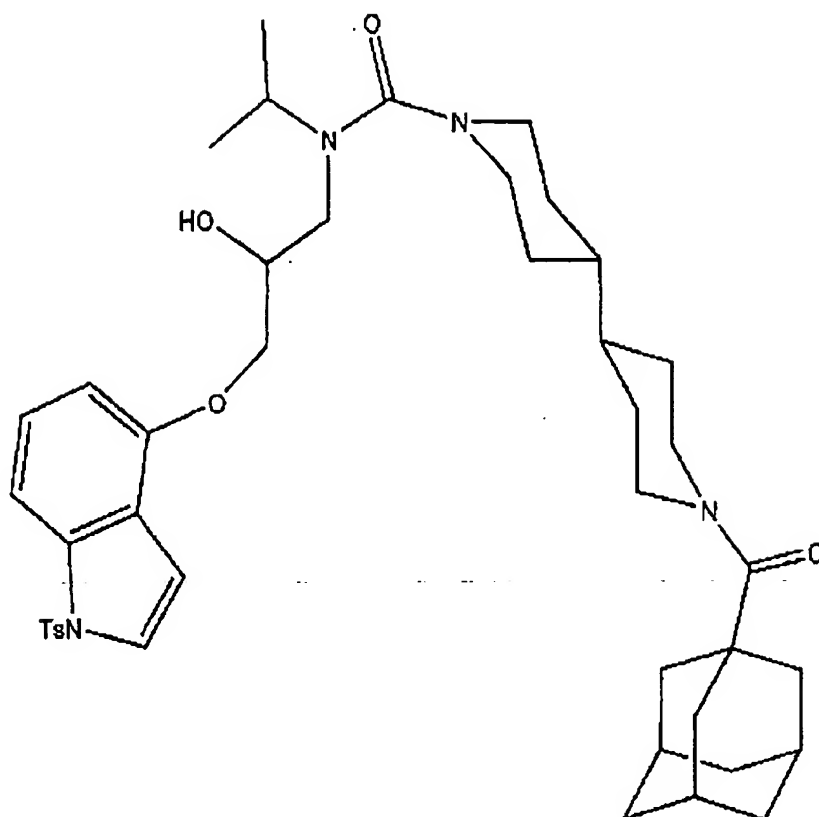
13. A compound of the formula:



14. A compound of the formula:



15. A compound of the formula:



16. A method of treating cancer in a mammal wherein a clinically effective amount of a small molecule inhibitor of Her2 expression is administered to said mammal.
17. The method of claim 16, wherein said cancer is breast cancer.
18. A method of treating breast cancer in a mammal, wherein a clinically effective amount of adamanolol, a functionalized adamanolol derivative, or a pharmaceutically acceptable salt thereof is administered to said mammal.
19. A pharmaceutical composition comprising the compound of claim 1 or pharmaceutically acceptable salts thereof.

1/37

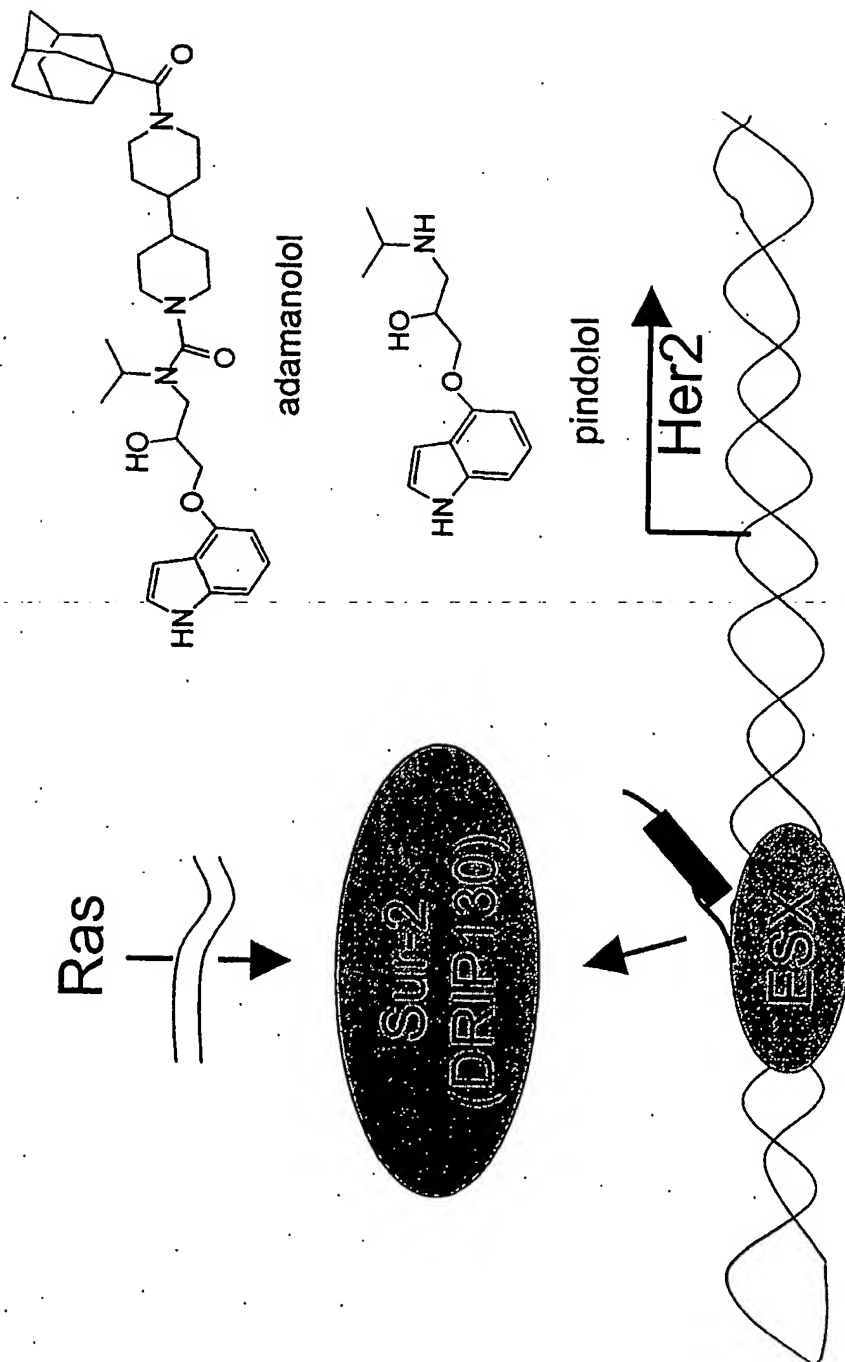


FIG. 1

2/37

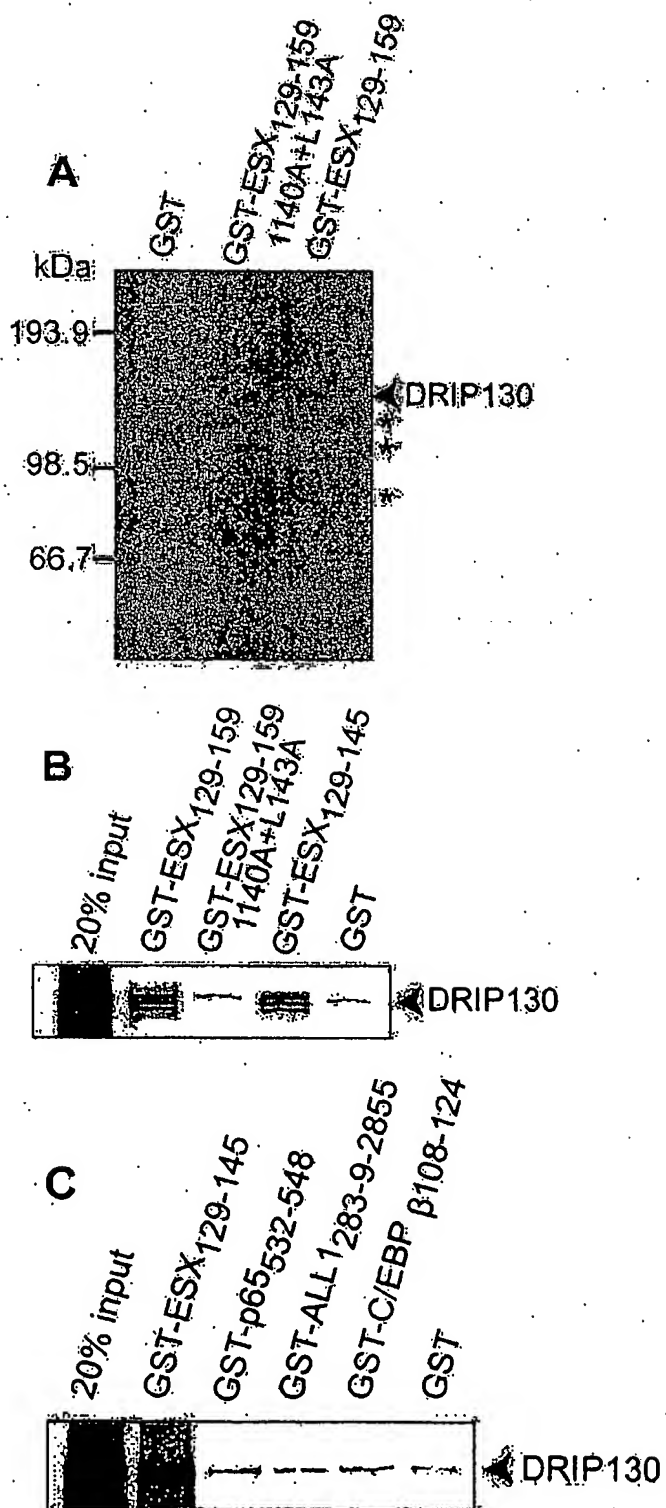


FIG. 2

3/37

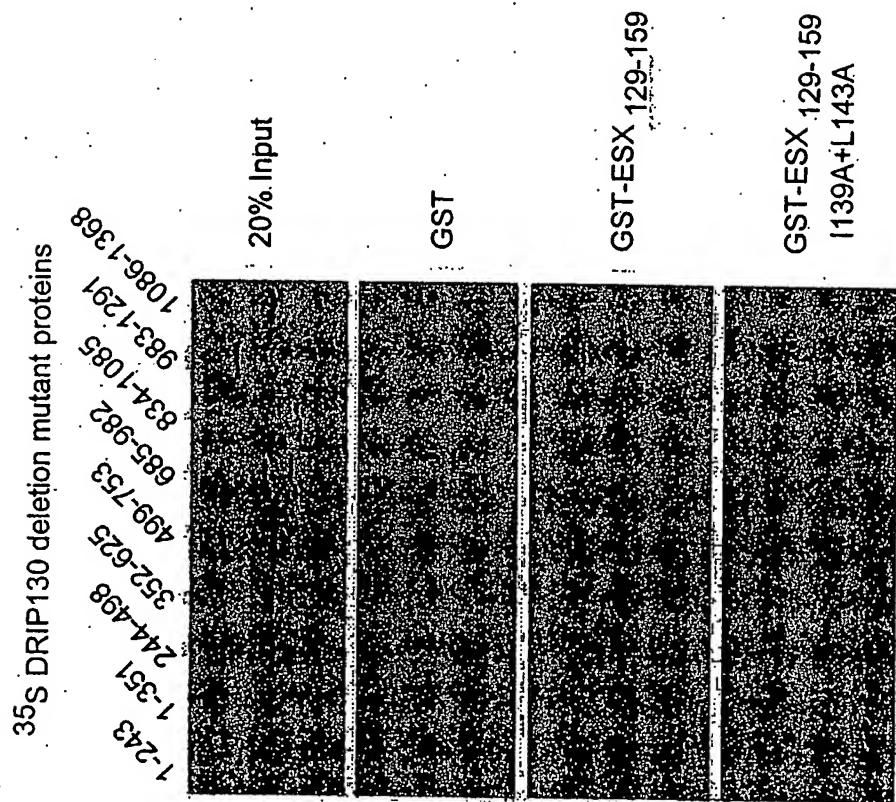


FIG. 3A

4/37

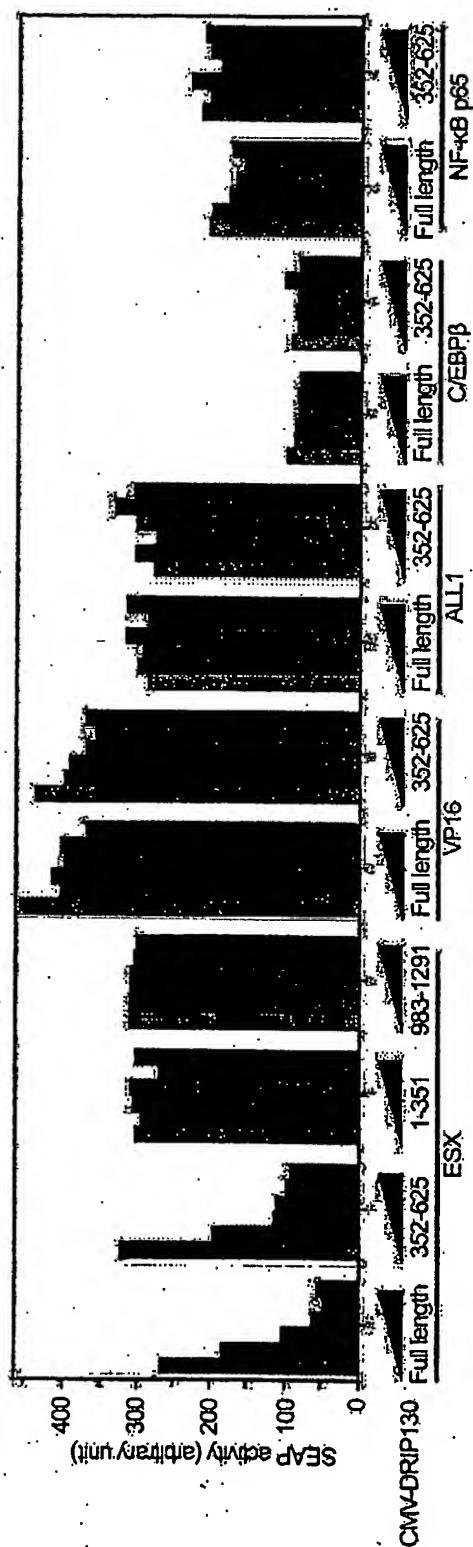


FIG. 3B

5/37

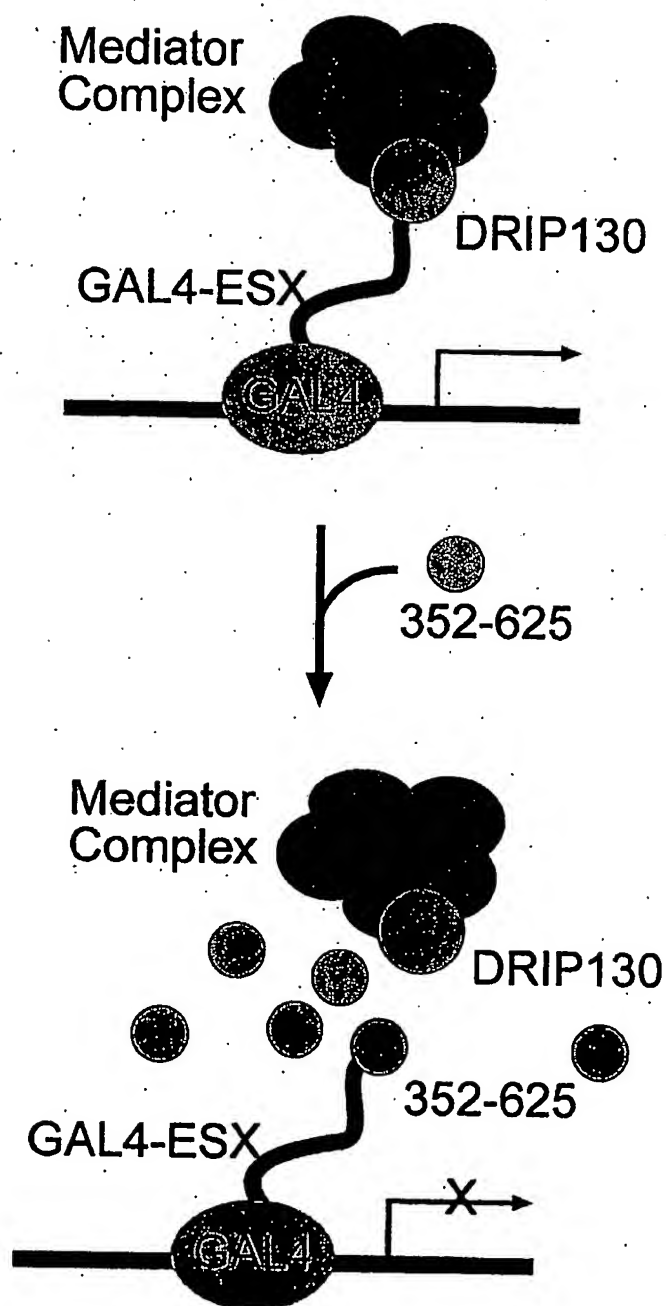


FIG. 3C

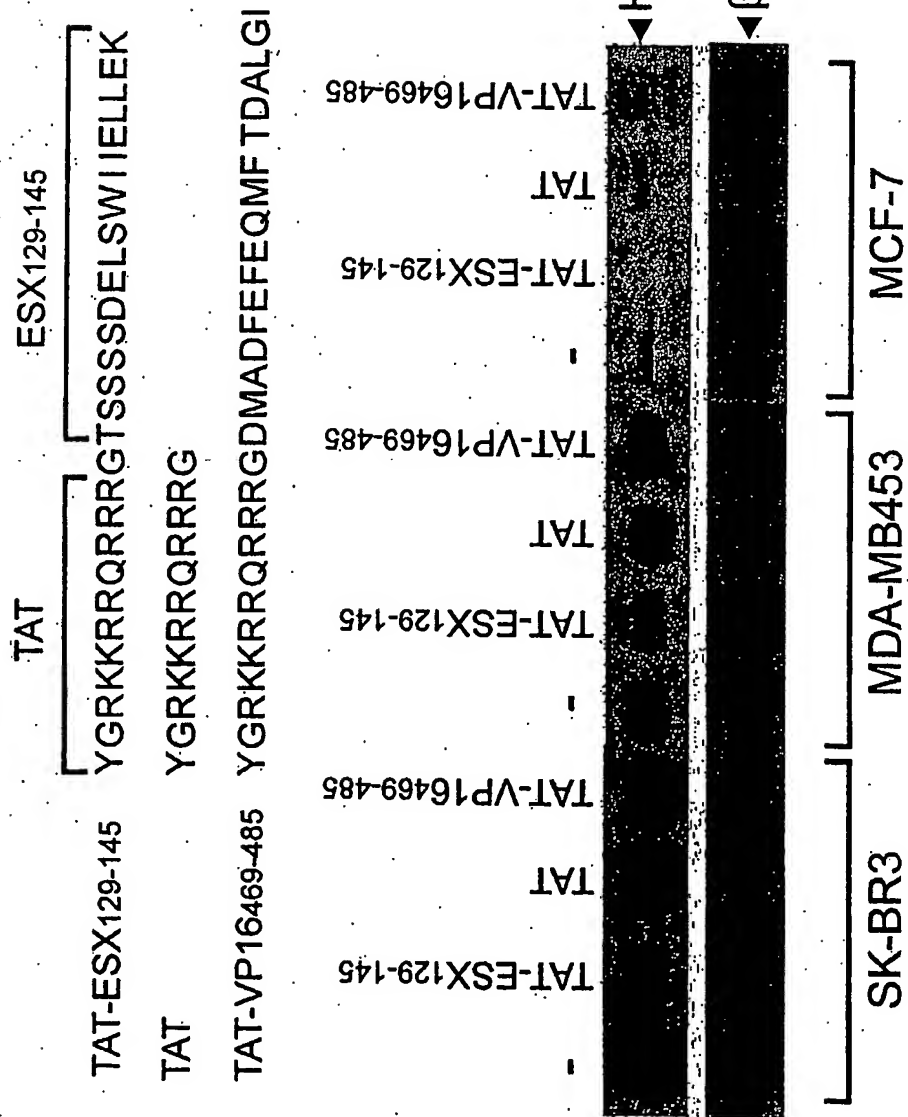


FIG. 4

7/37

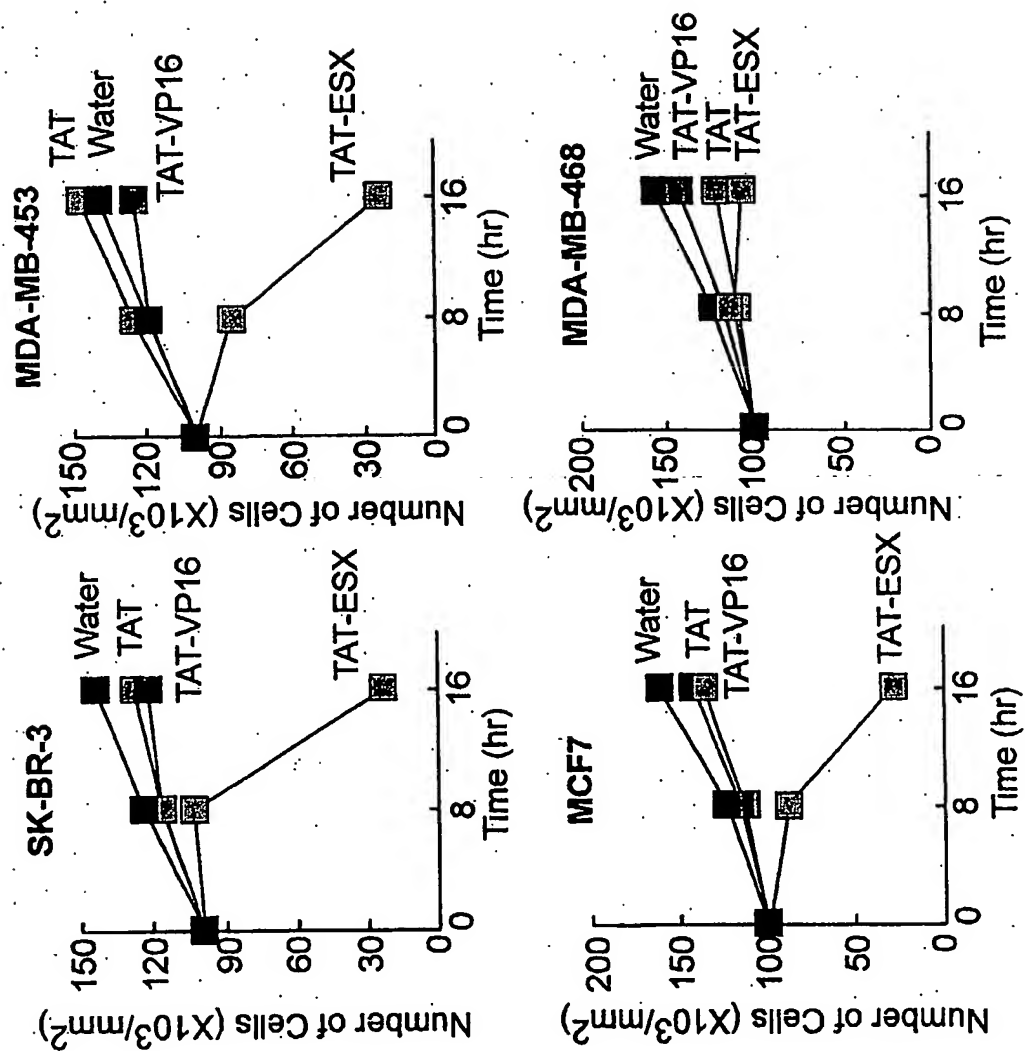


FIG. 5

8/37

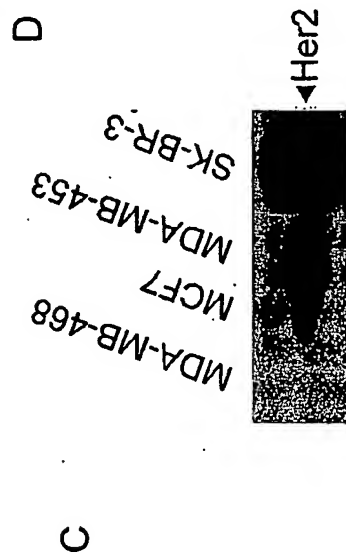
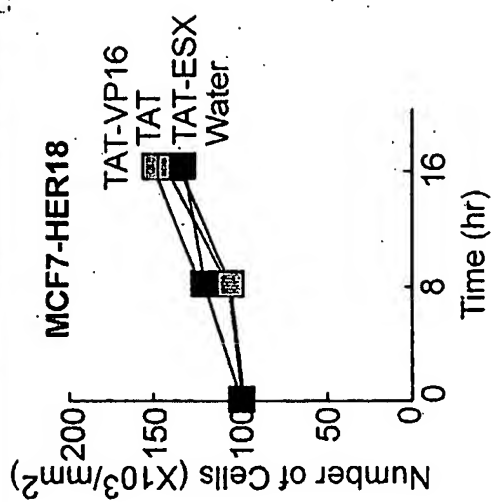
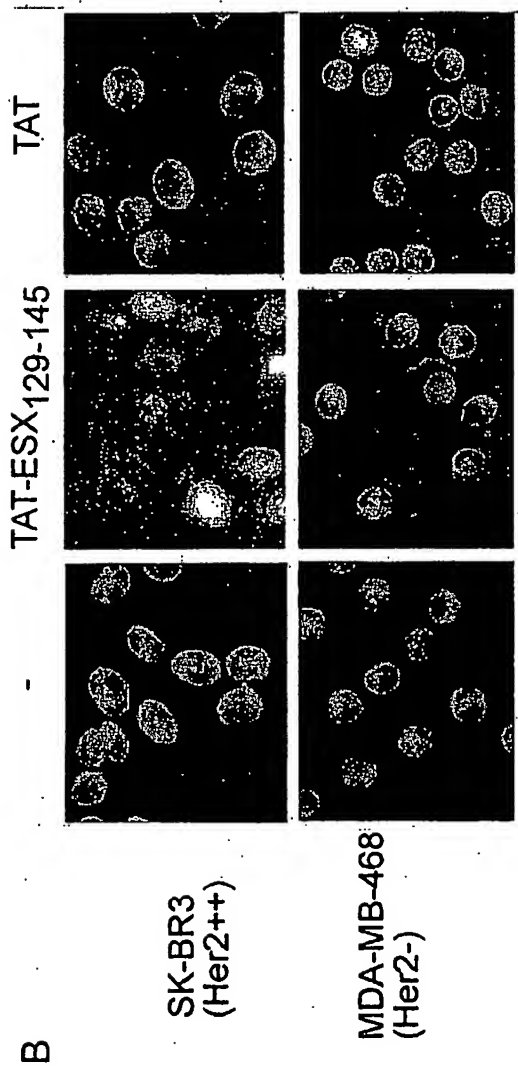


FIG. 5

9/37

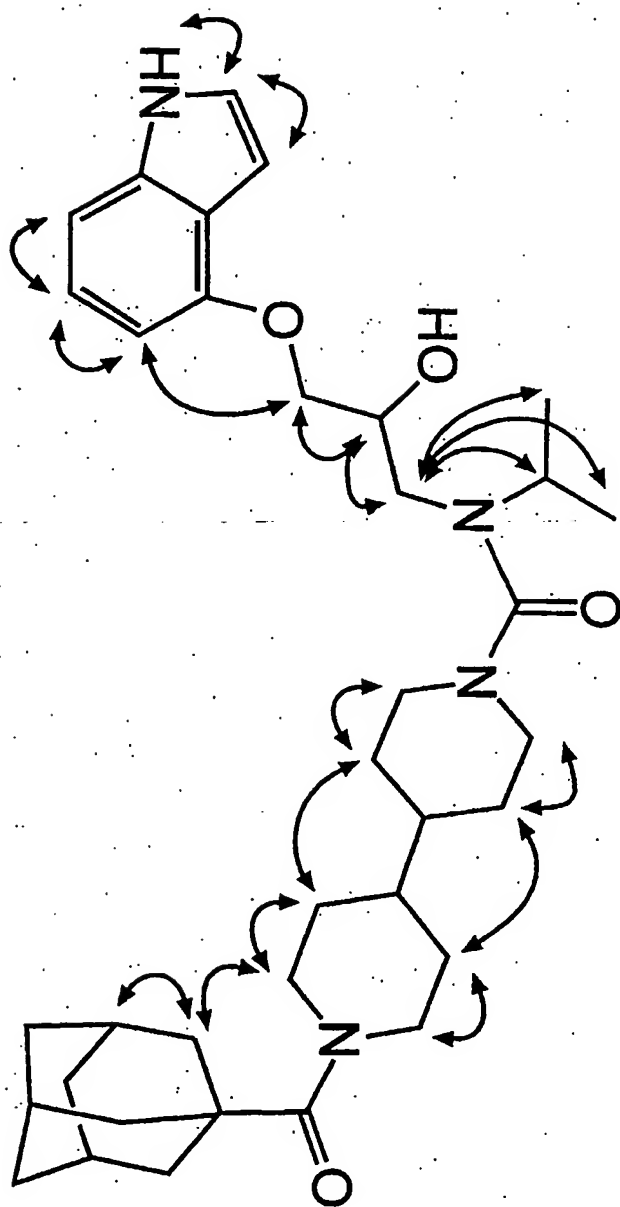


FIG. 6

10/37

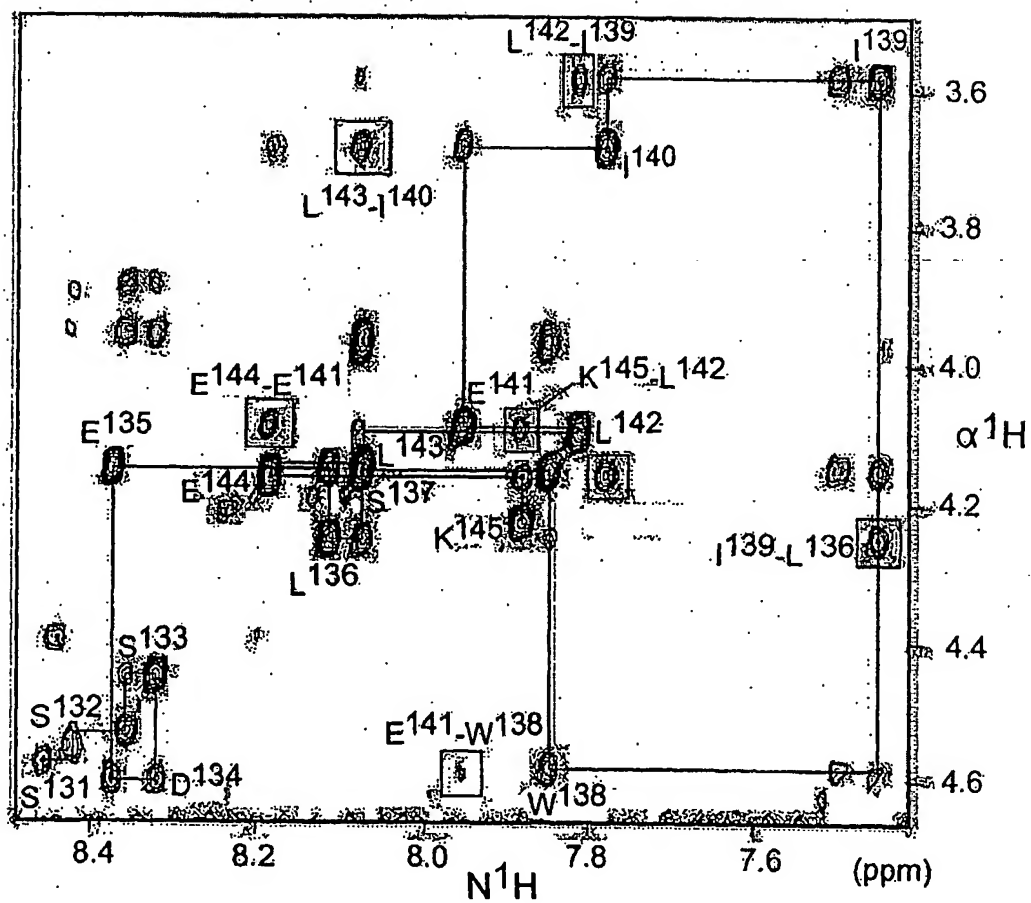


FIG. 7A

11/37

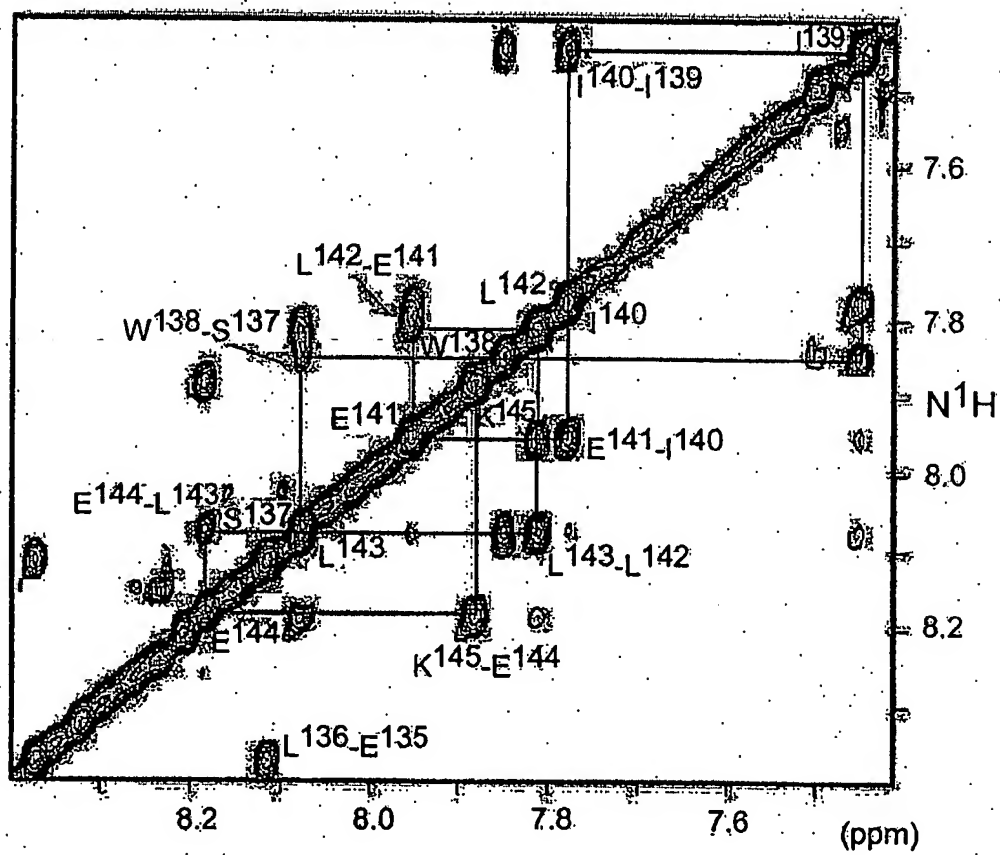


FIG. 7B

12/37

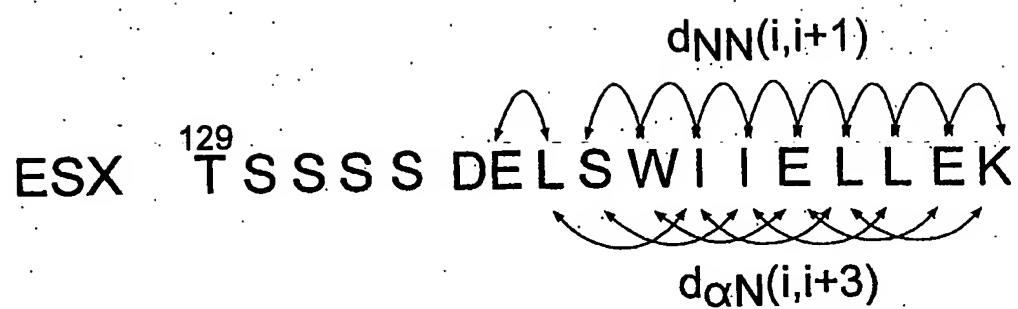


FIG. 7C

13/37

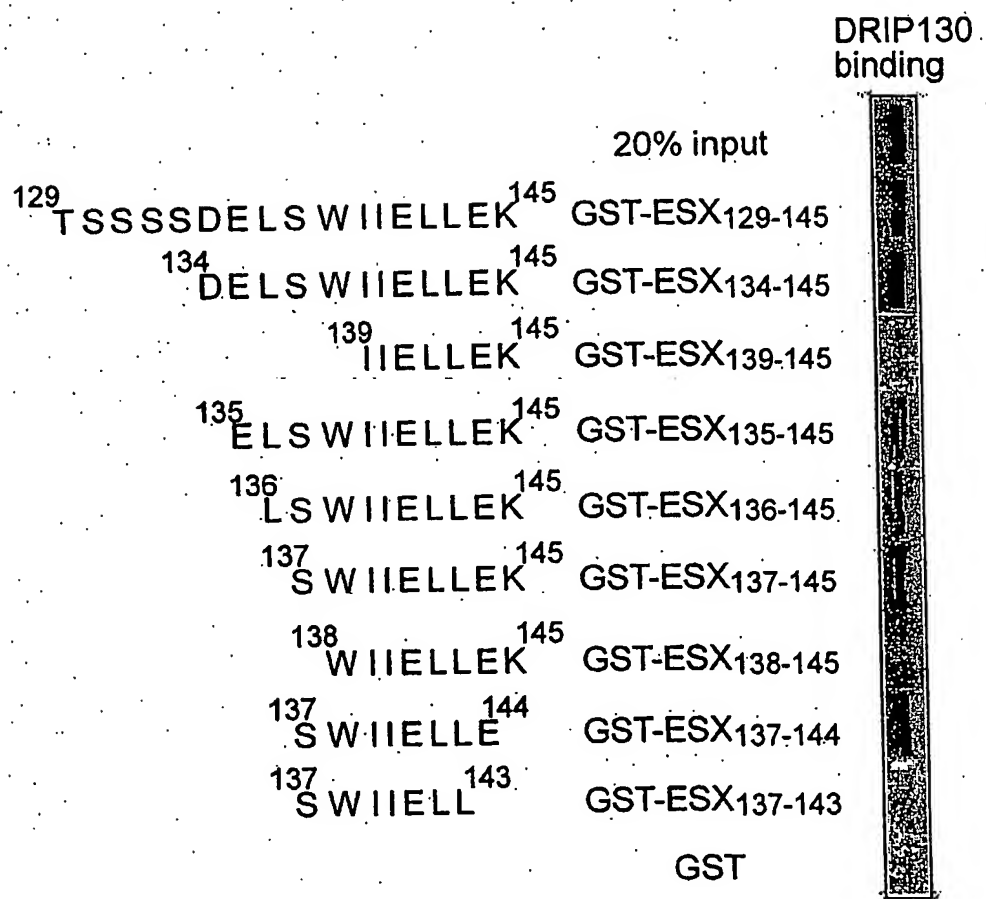


FIG. 8A

14/37

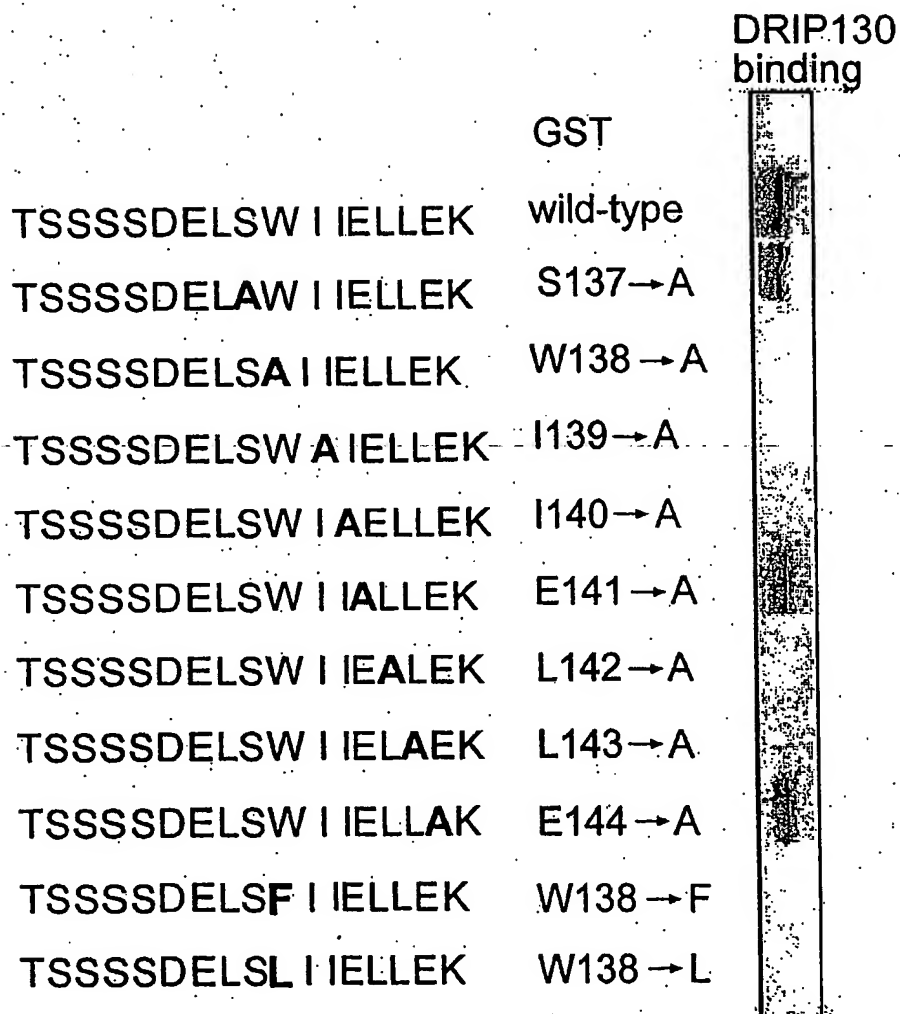


FIG. 8B

15/37

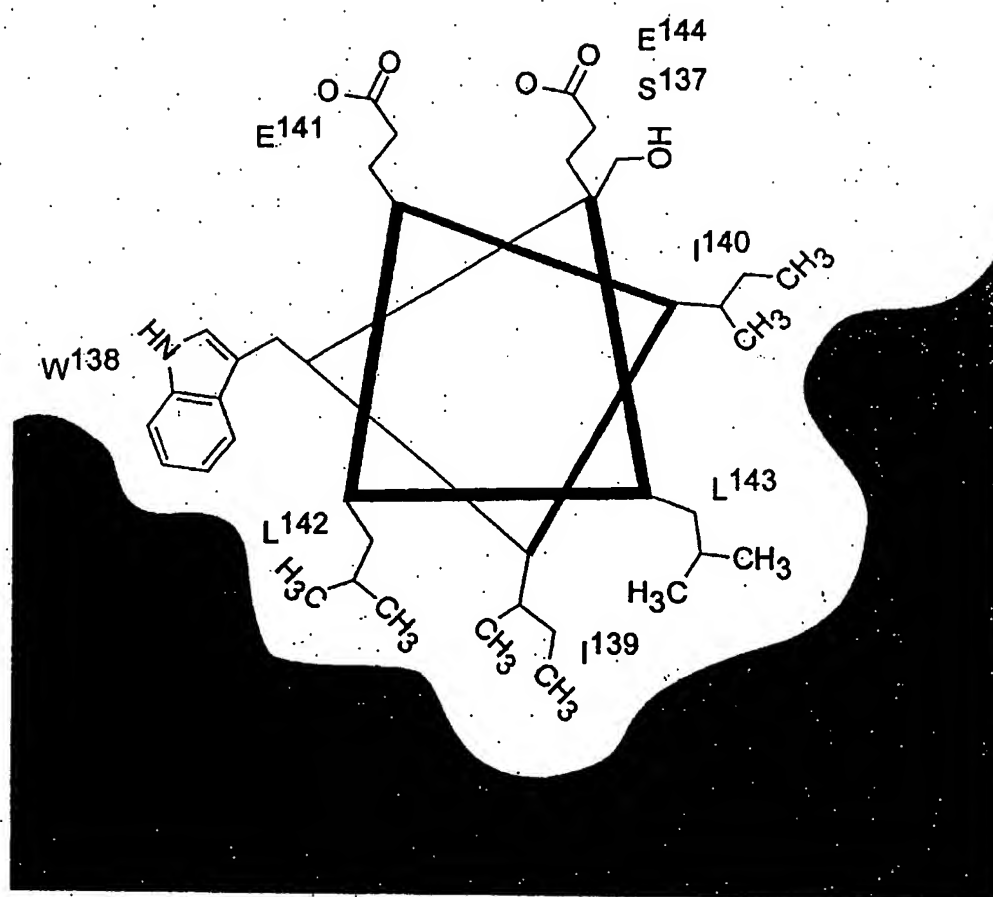


FIG. 8C

16/37

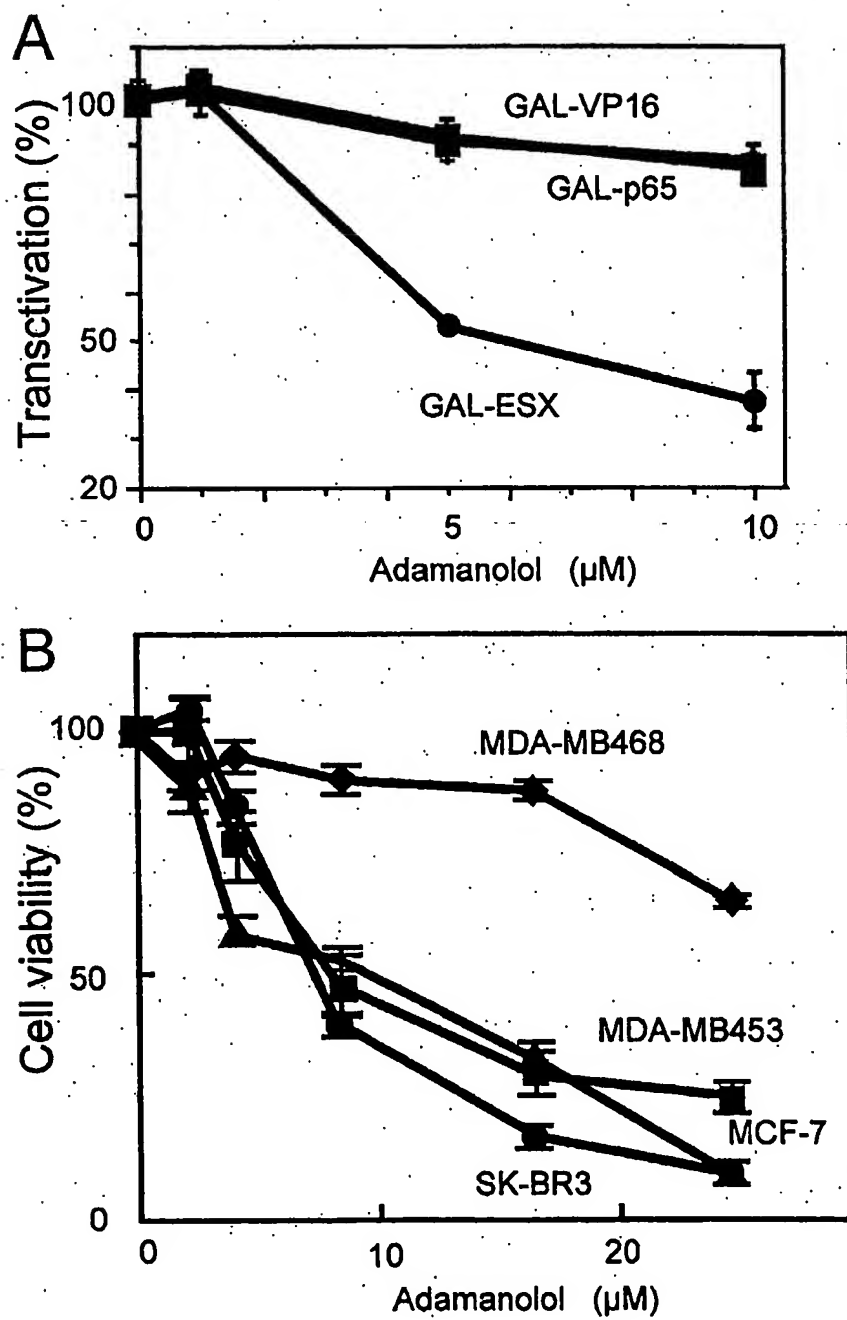
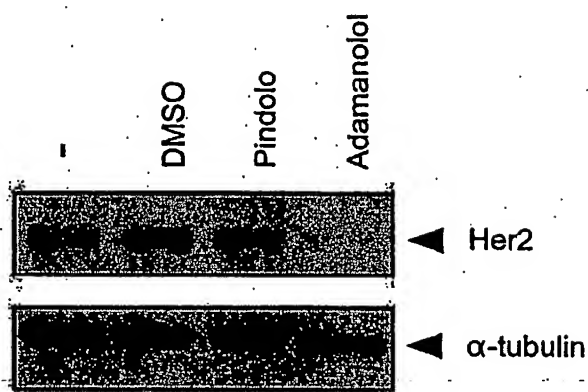


FIG. 9

17/37

C



D

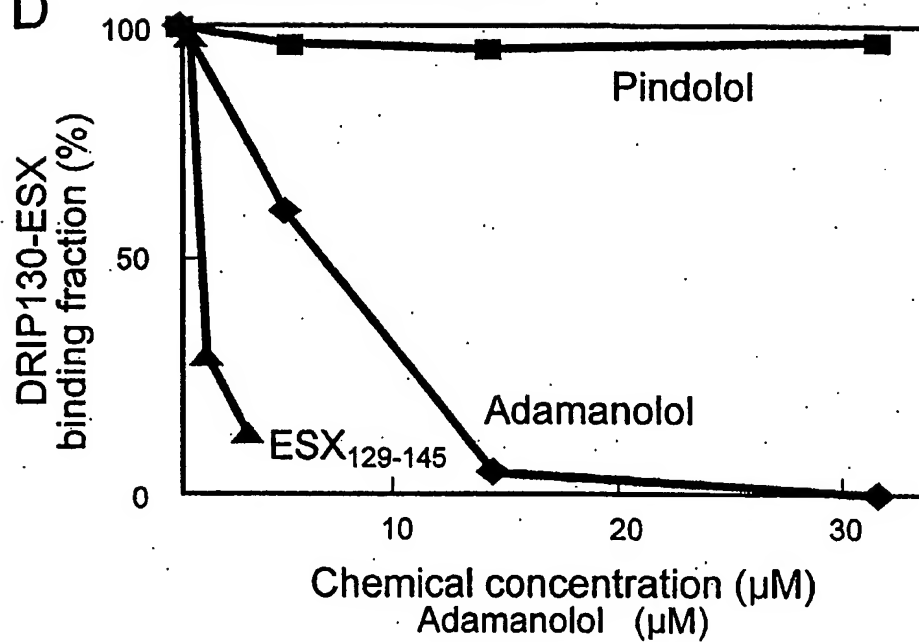


FIG. 9

18/37

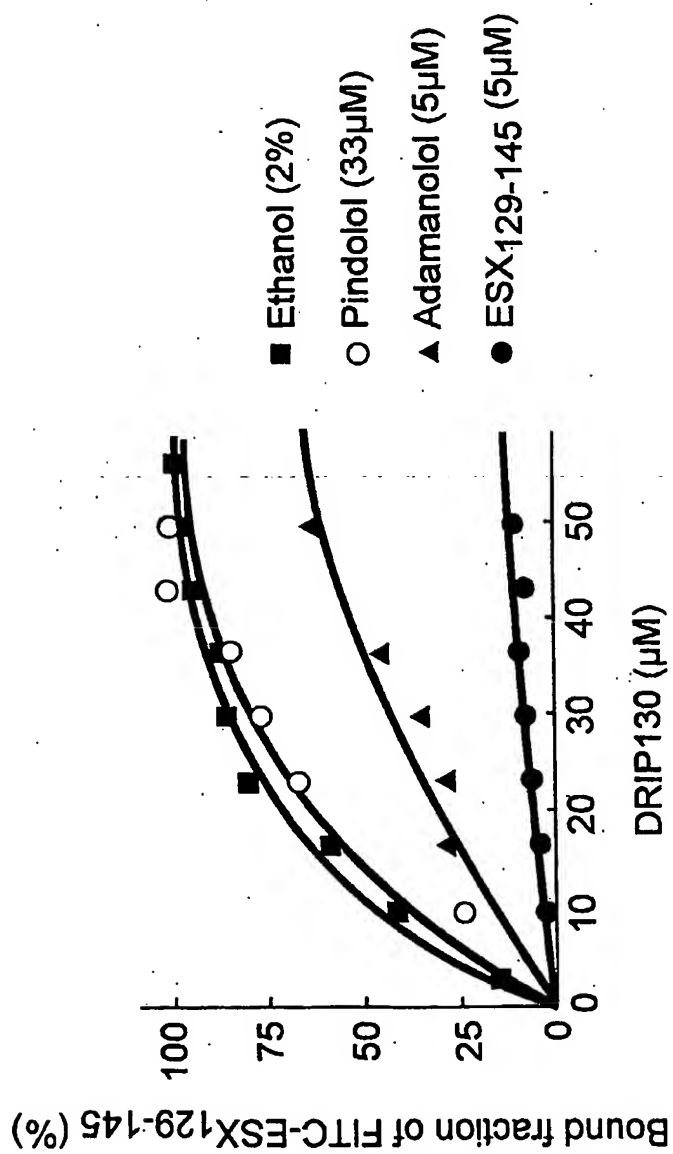


FIG. 10

19/37

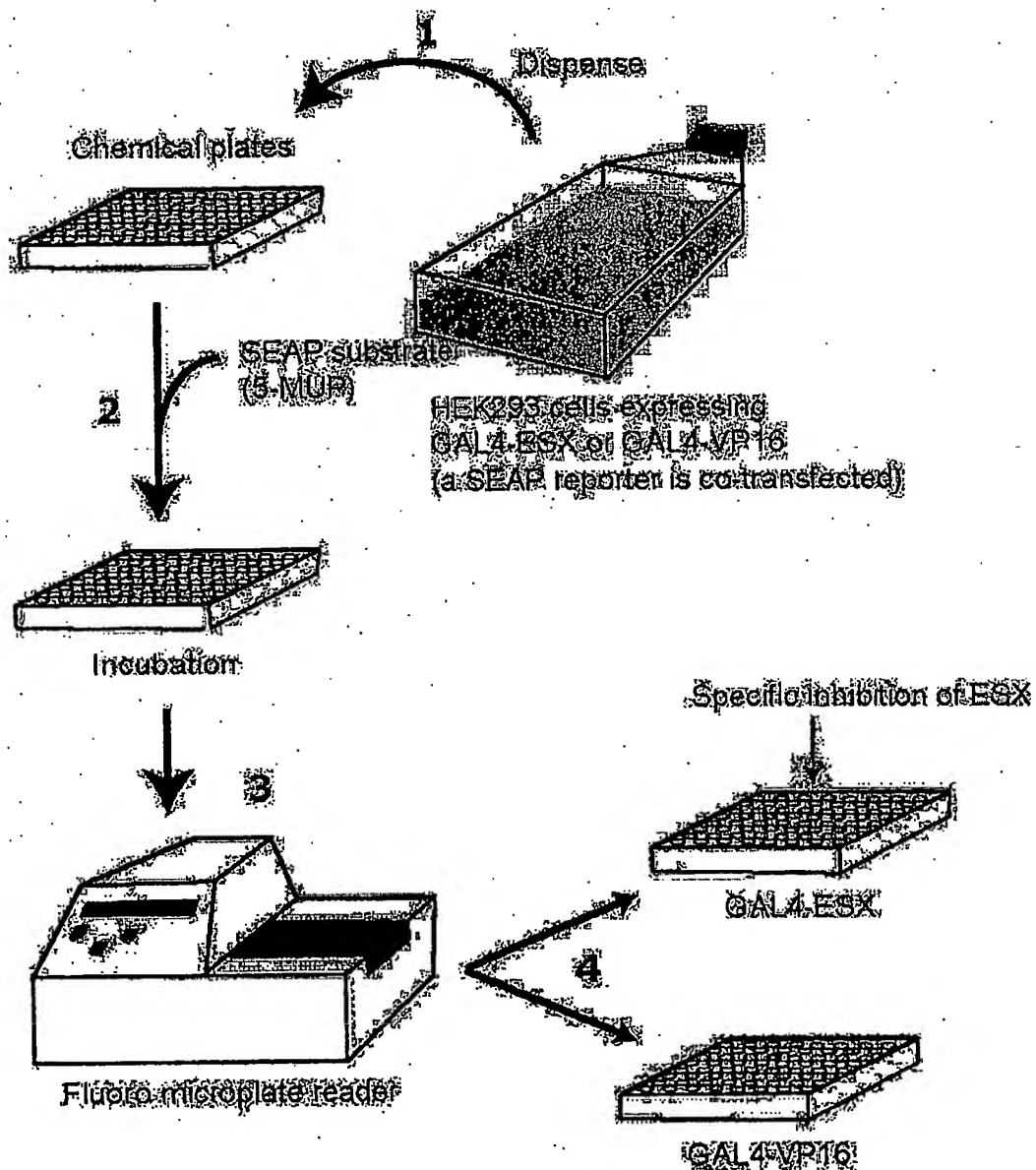
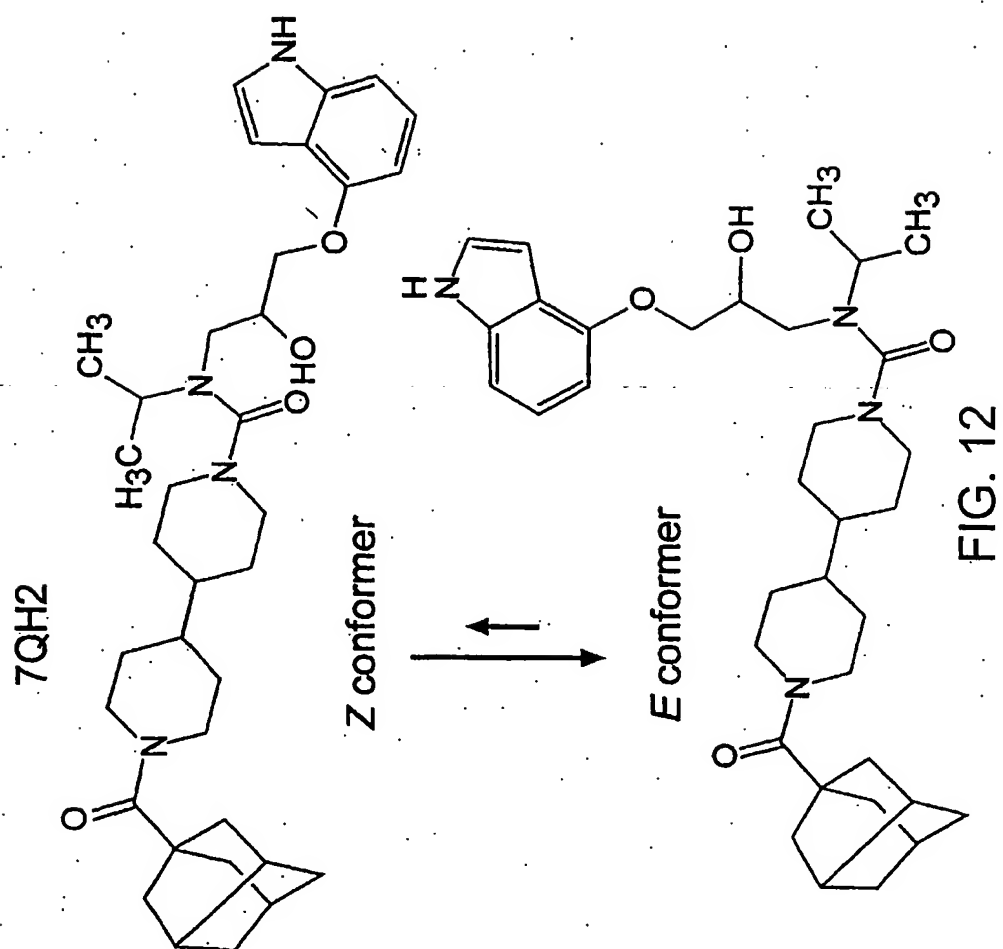


FIG. 11

20/37



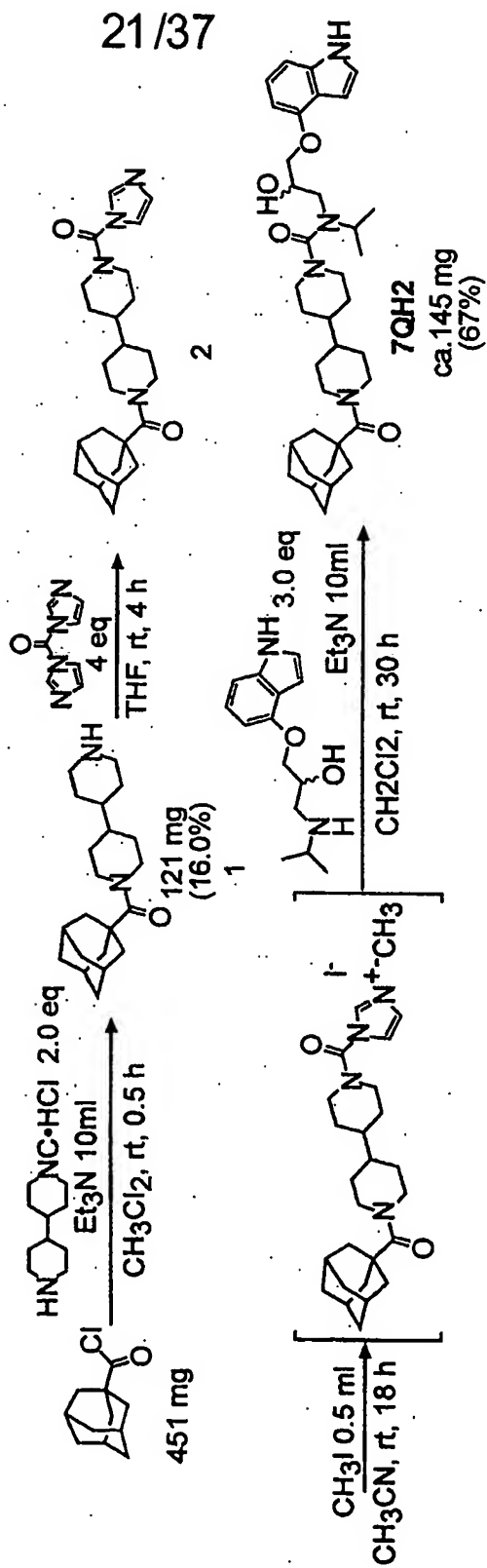


FIG. 13

22/37

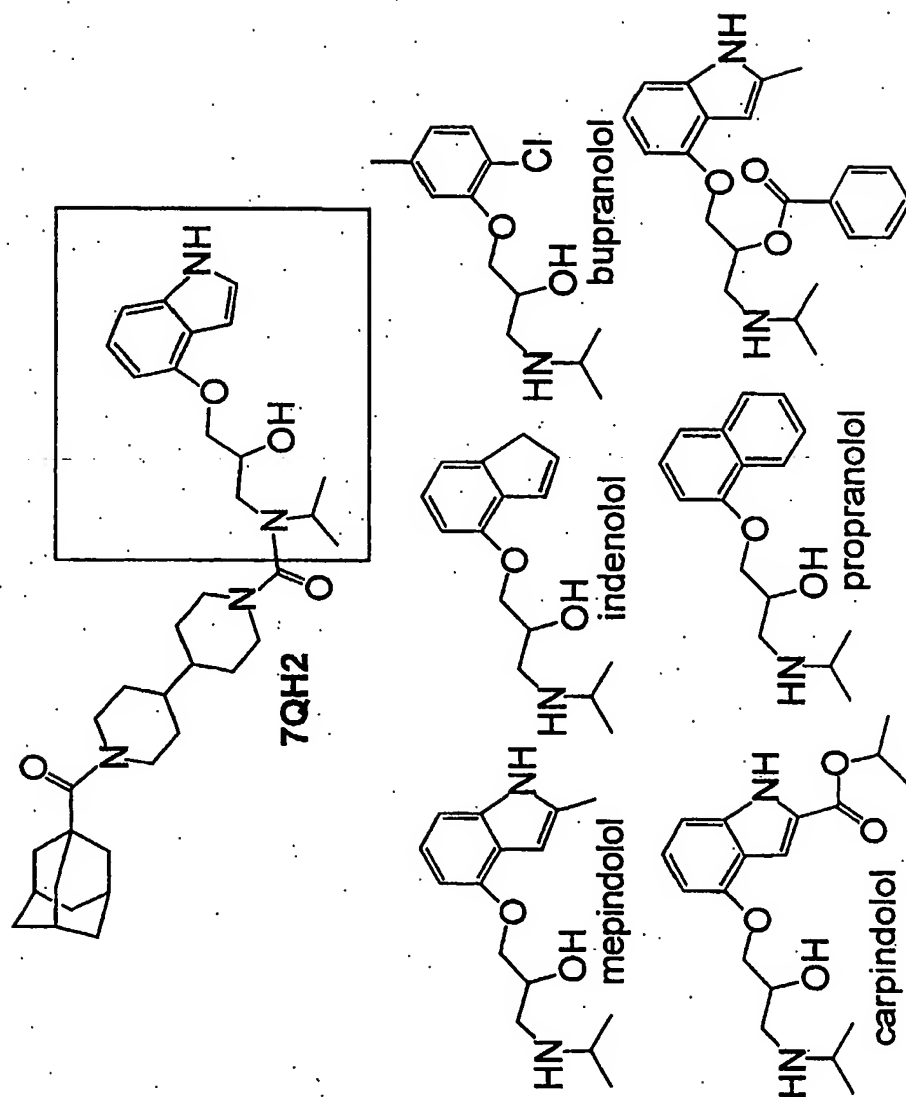


FIG. 14

23/37

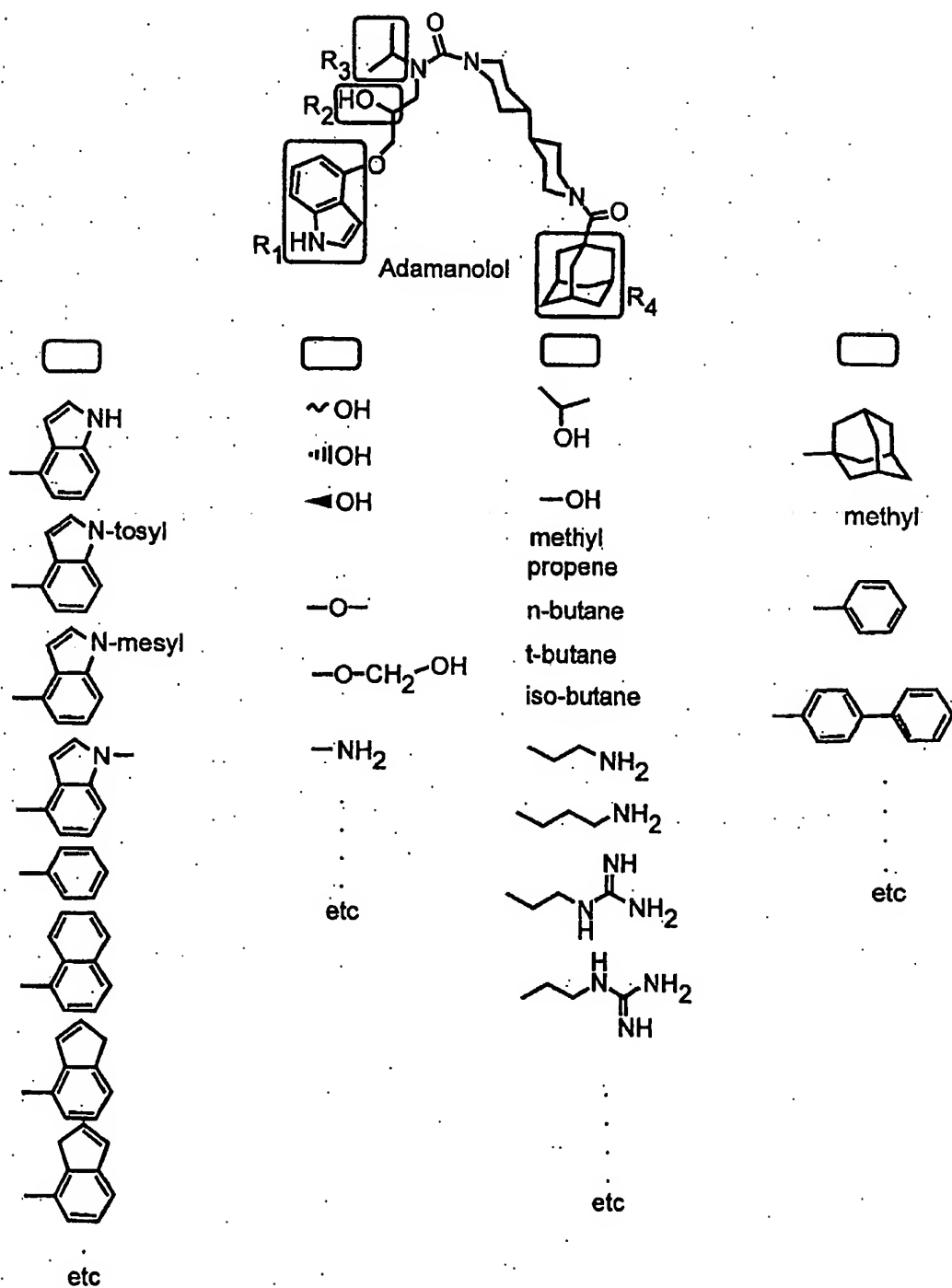


FIG. 15

24/37

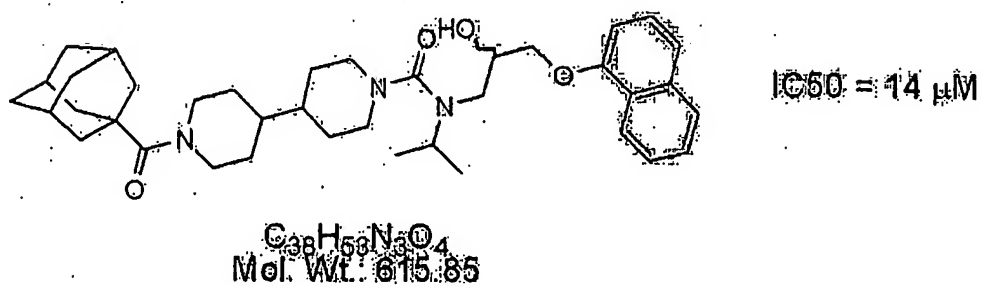
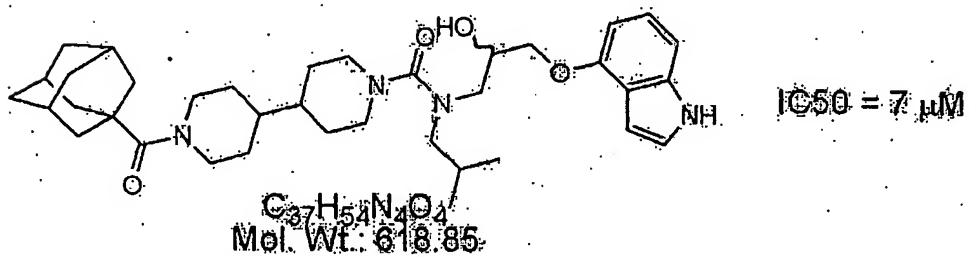
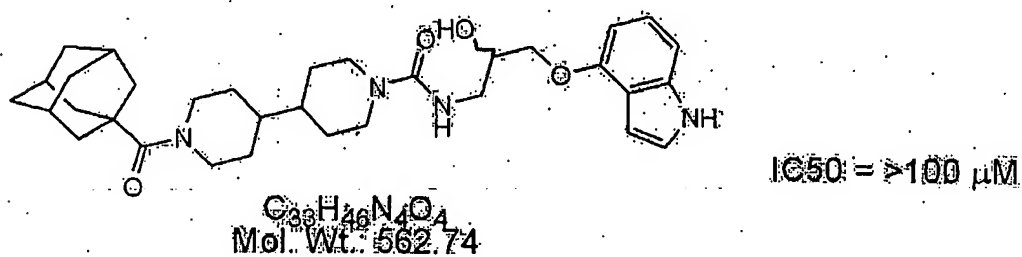
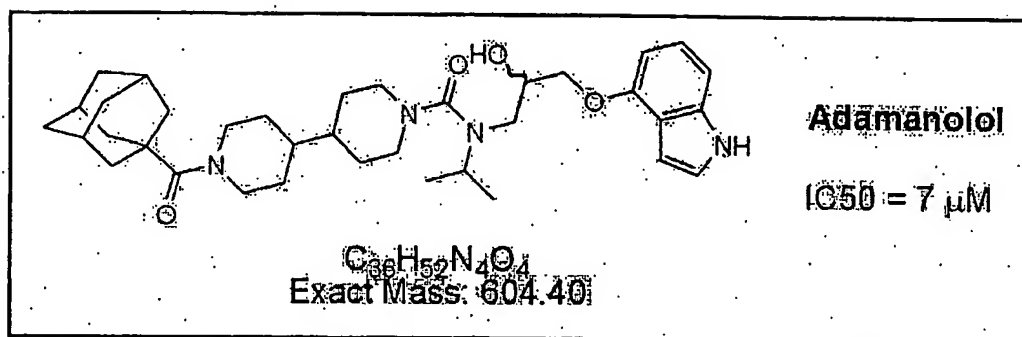


FIG. 16A

25/37

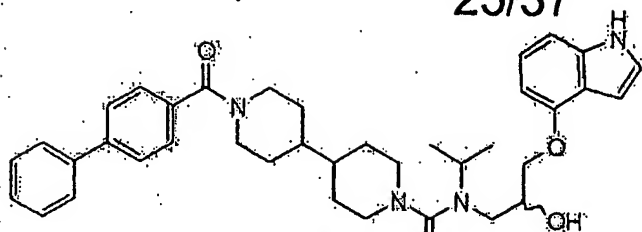
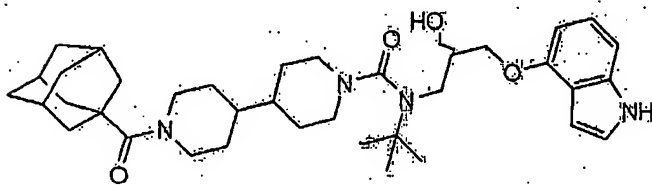
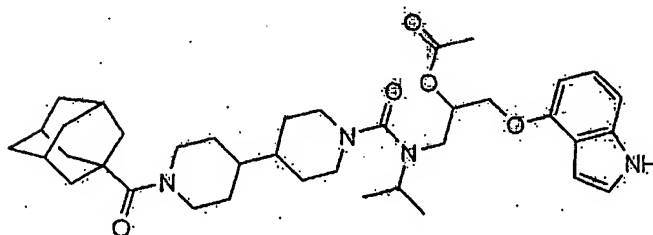
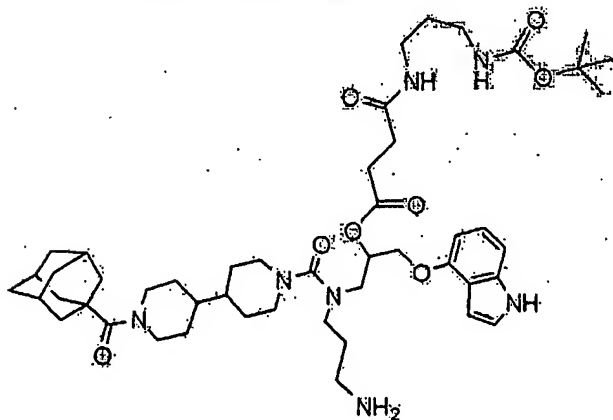
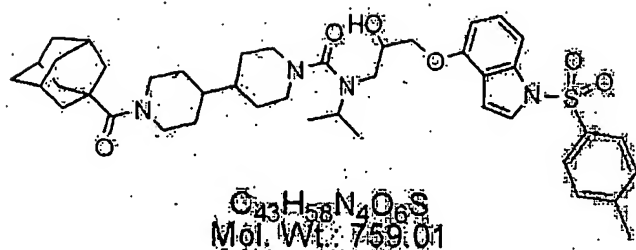
IC₅₀ = 20 μ M $C_{38}H_{48}N_4O_4$
Mol. Wt.: 622.80IC₅₀ = 14 μ M $C_{37}H_{54}N_4O_4$
Exact Mass: 618.41IC₅₀ = >100 μ M $C_{38}H_{54}N_4O_5$
Exact Mass: 646.41IC₅₀ = >100 μ M $C_{48}H_{73}N_7O_8$
Exact Mass: 875.55

FIG. 16B

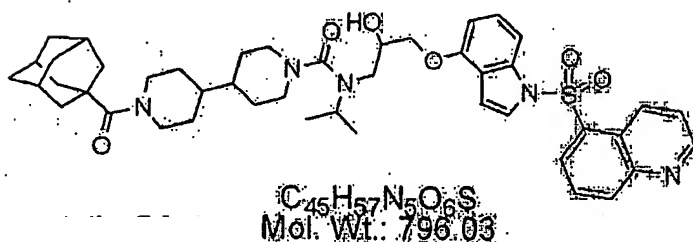
26/37



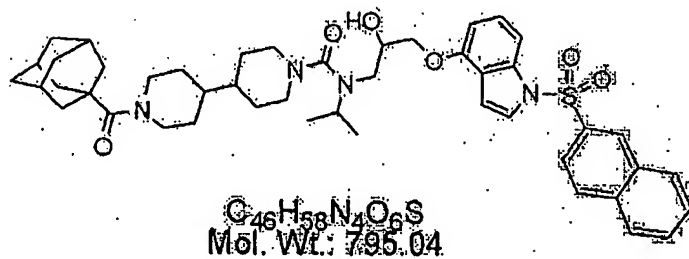
IC₅₀ = 3 μM

$$\text{C}_{43}\text{H}_{58}\text{N}_4\text{O}_6\text{S}$$

Mol. Wt.: 759.01

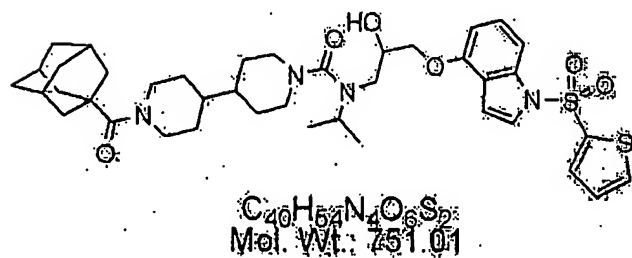

$$IC_{50} = 7 \mu M$$
$$\text{C}_{45}\text{H}_{57}\text{N}_5\text{O}_6\text{S}$$

Mol. Wt.: 796.03



IC50 = 7 μ M

C₄₆H₅₈N₄O₆S
Mol. Wt.: 795.04



IC50 = 7 μ M

$$\text{C}_{40}\text{H}_{54}\text{N}_4\text{O}_6\text{S}_2$$
Mol. Wt.: 751.01

FIG. 16C

27/37

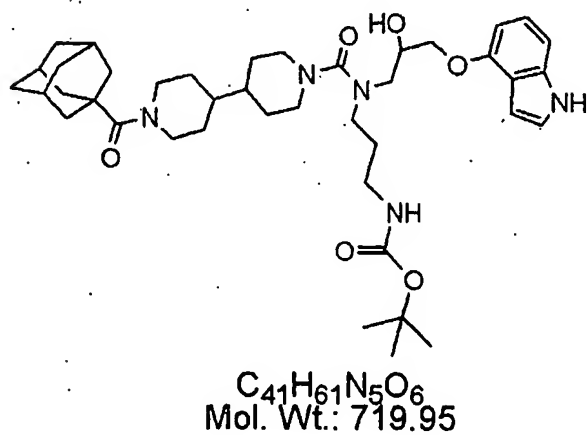
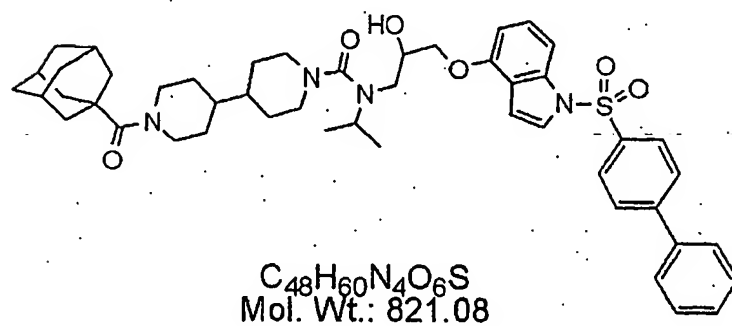
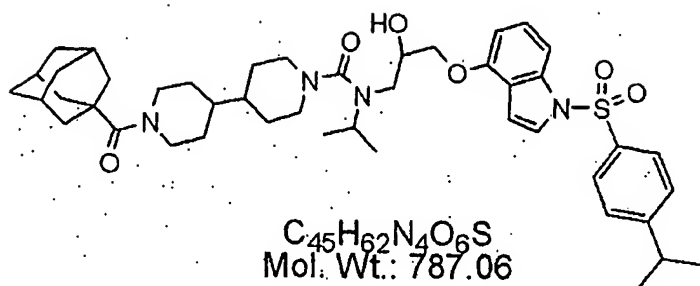
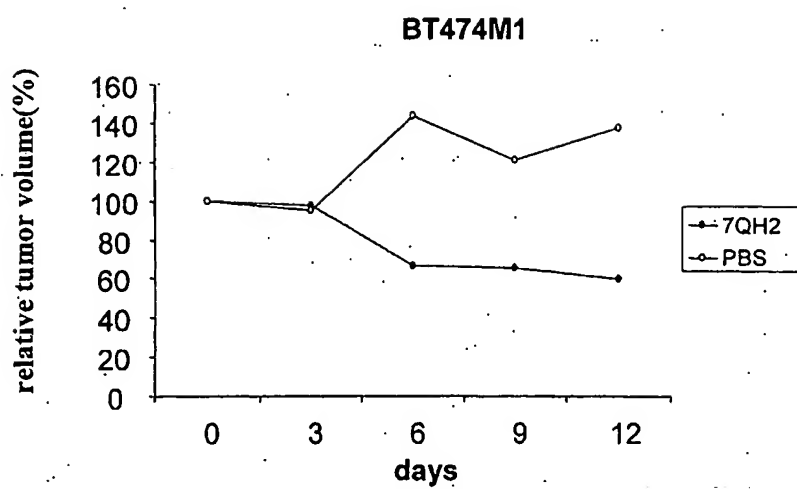


FIG. 16D

28/37

A



B

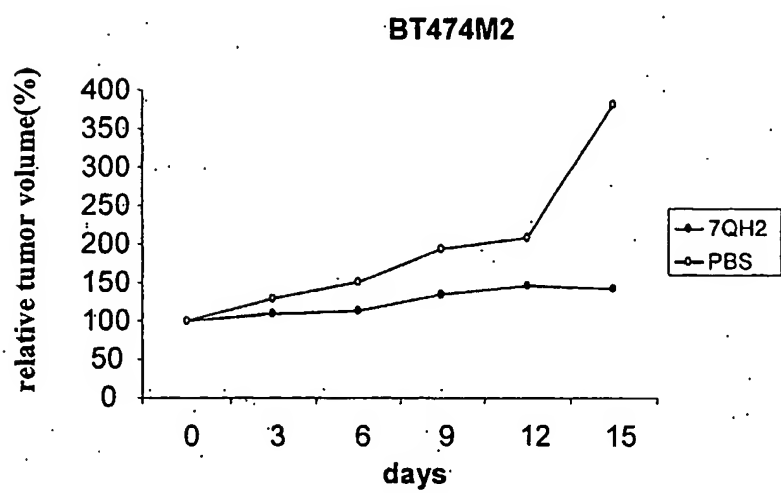


FIG. 17

29/37

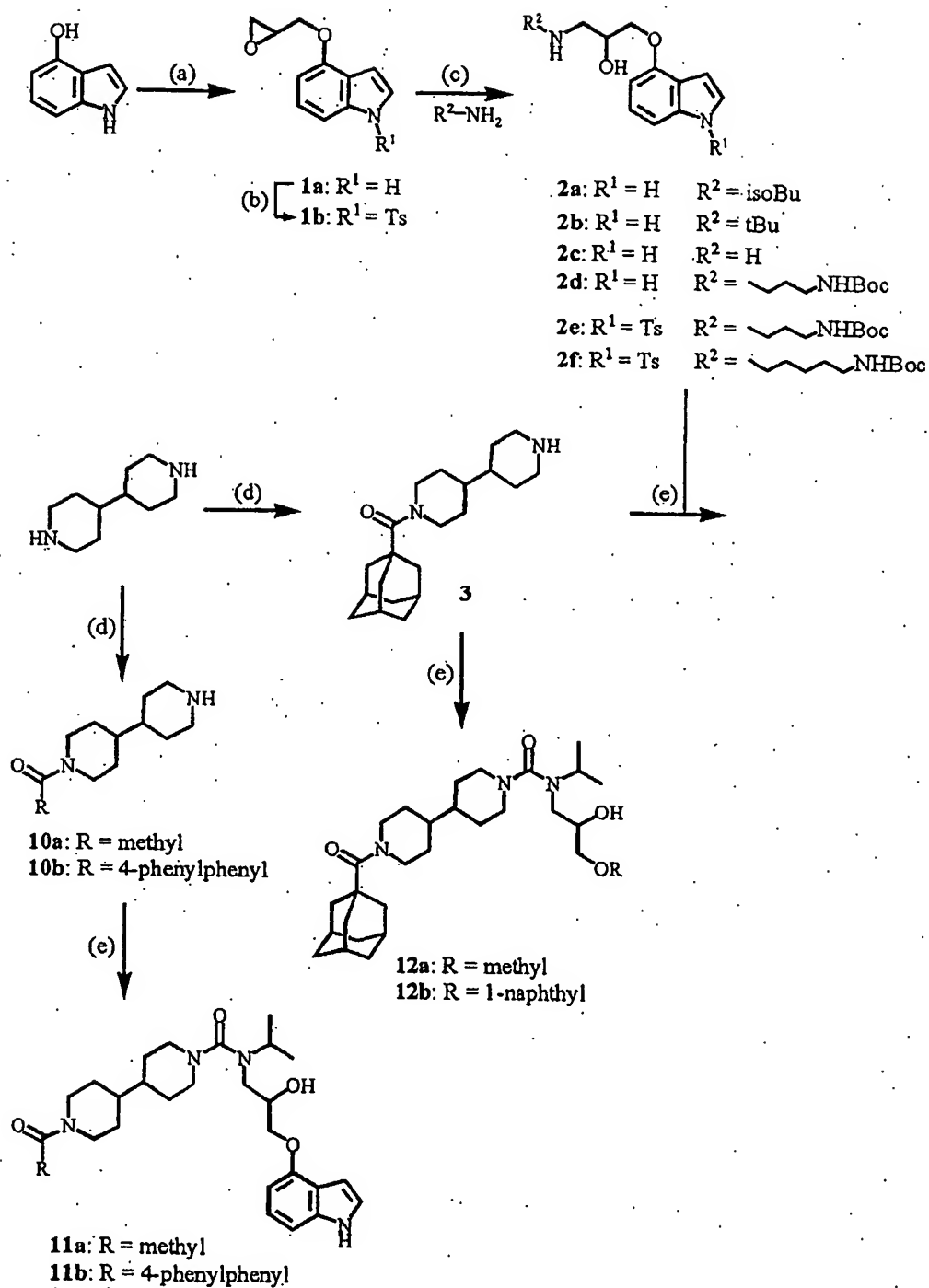


FIG. 18

30/37

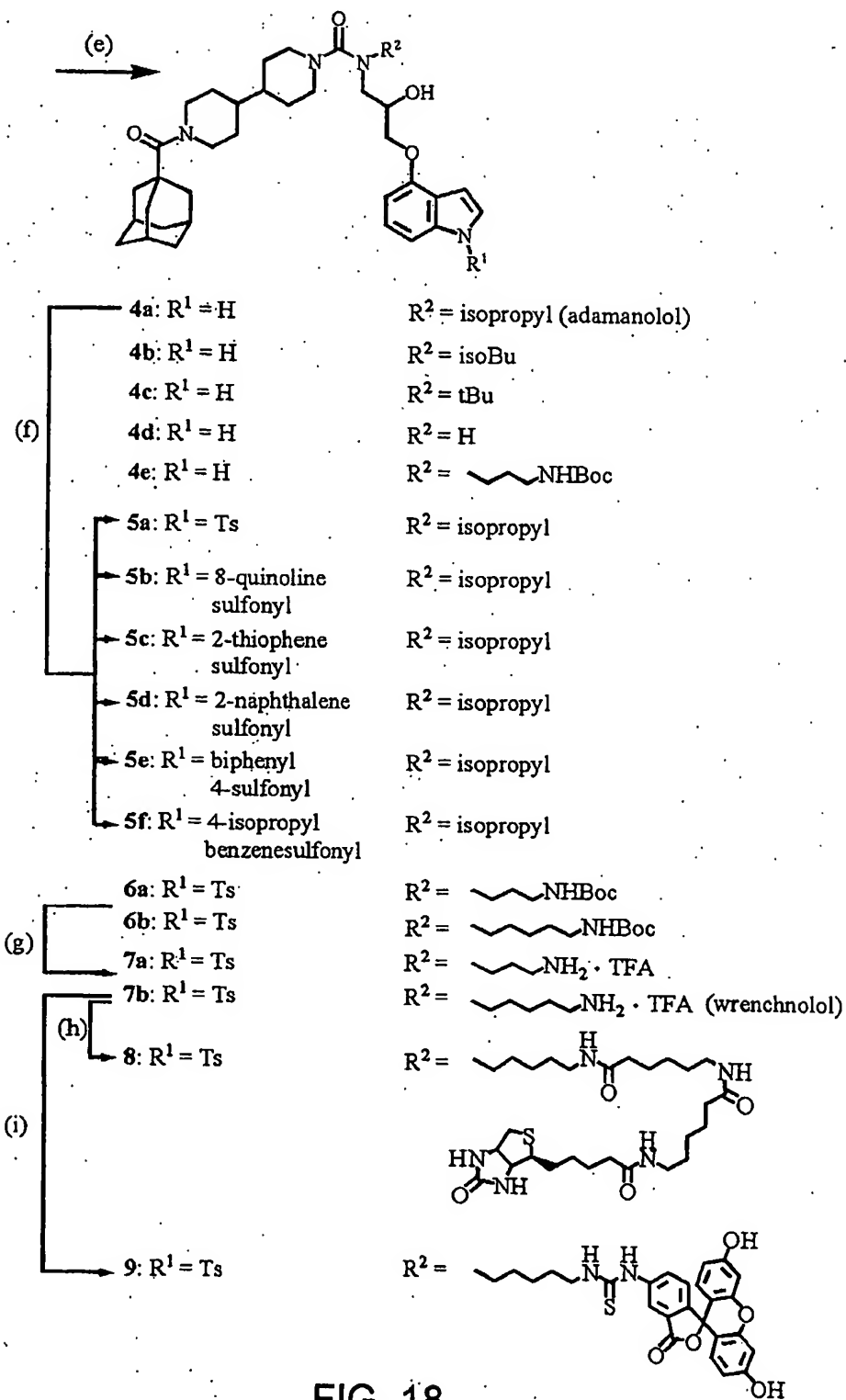


FIG. 18

31/37

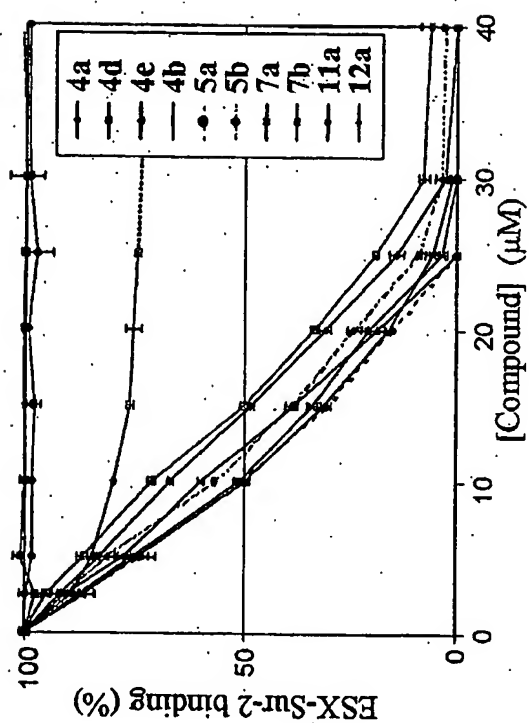
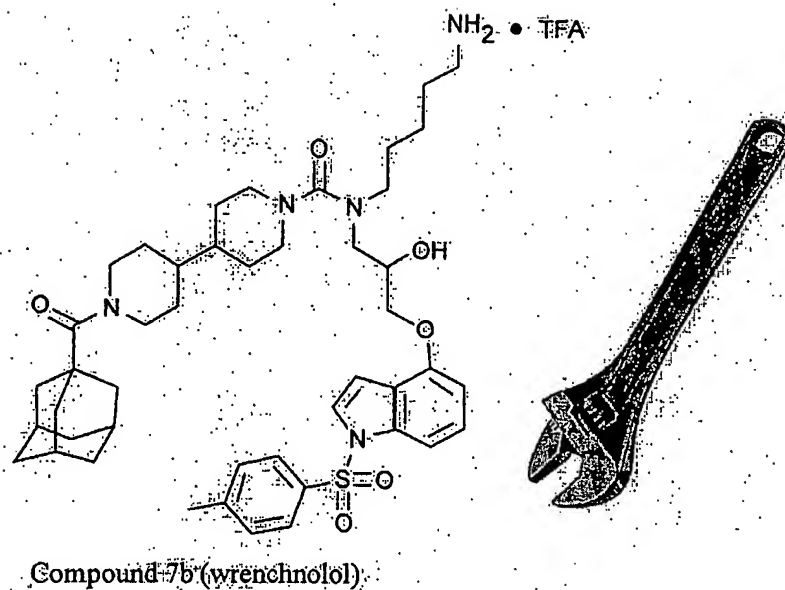


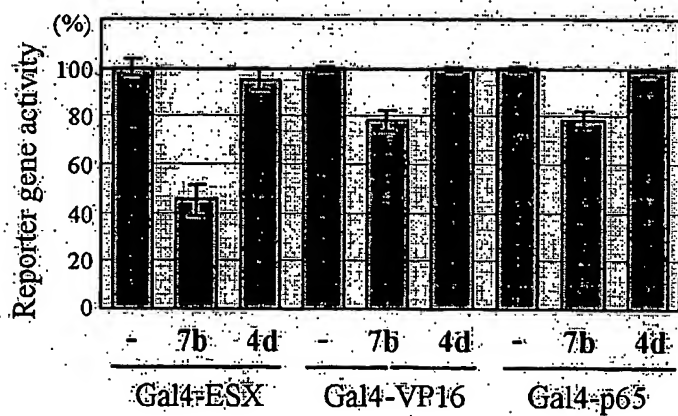
FIG. 19

32/37

A



B



C

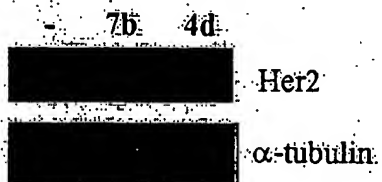


FIG. 20

33/37

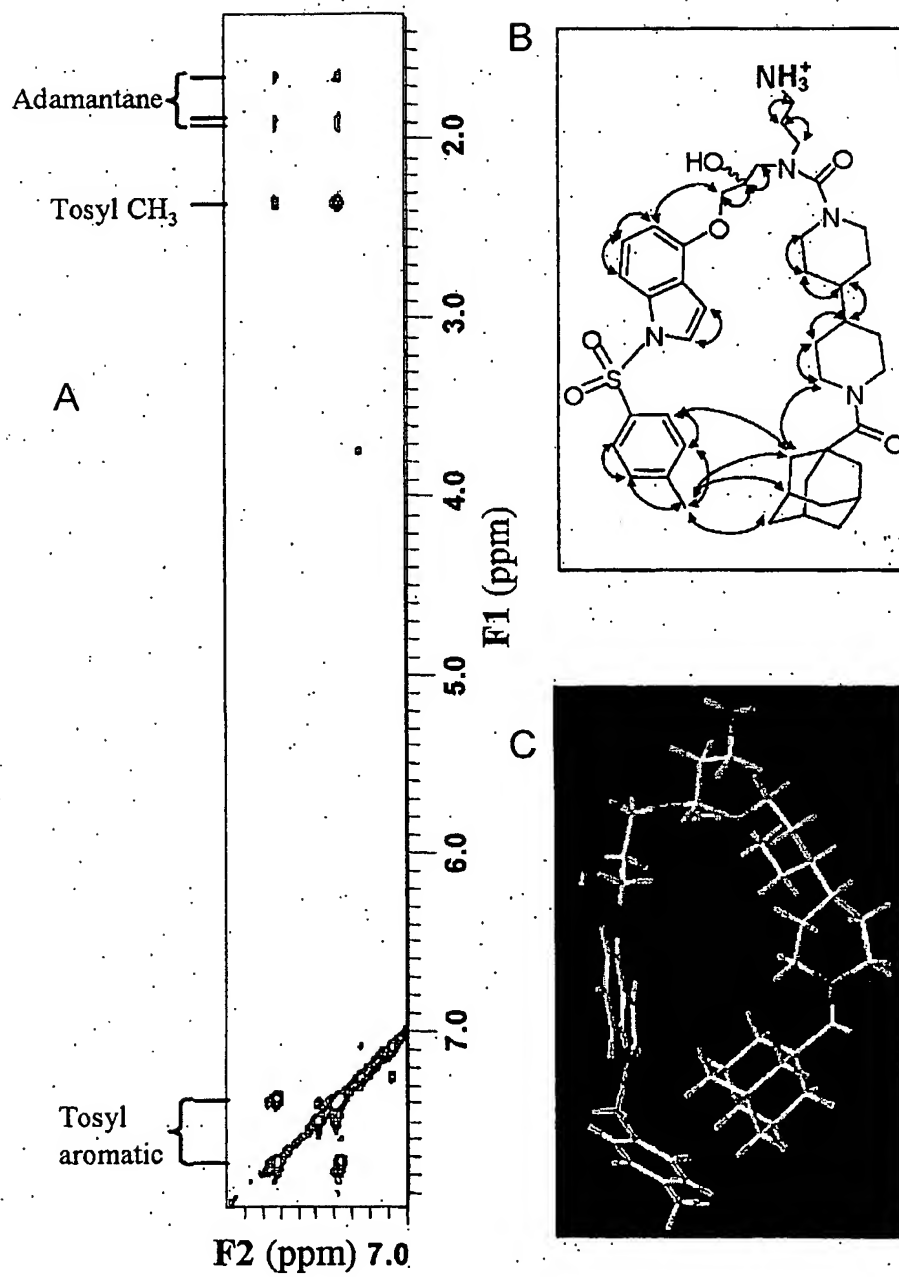


FIG. 21

34/37

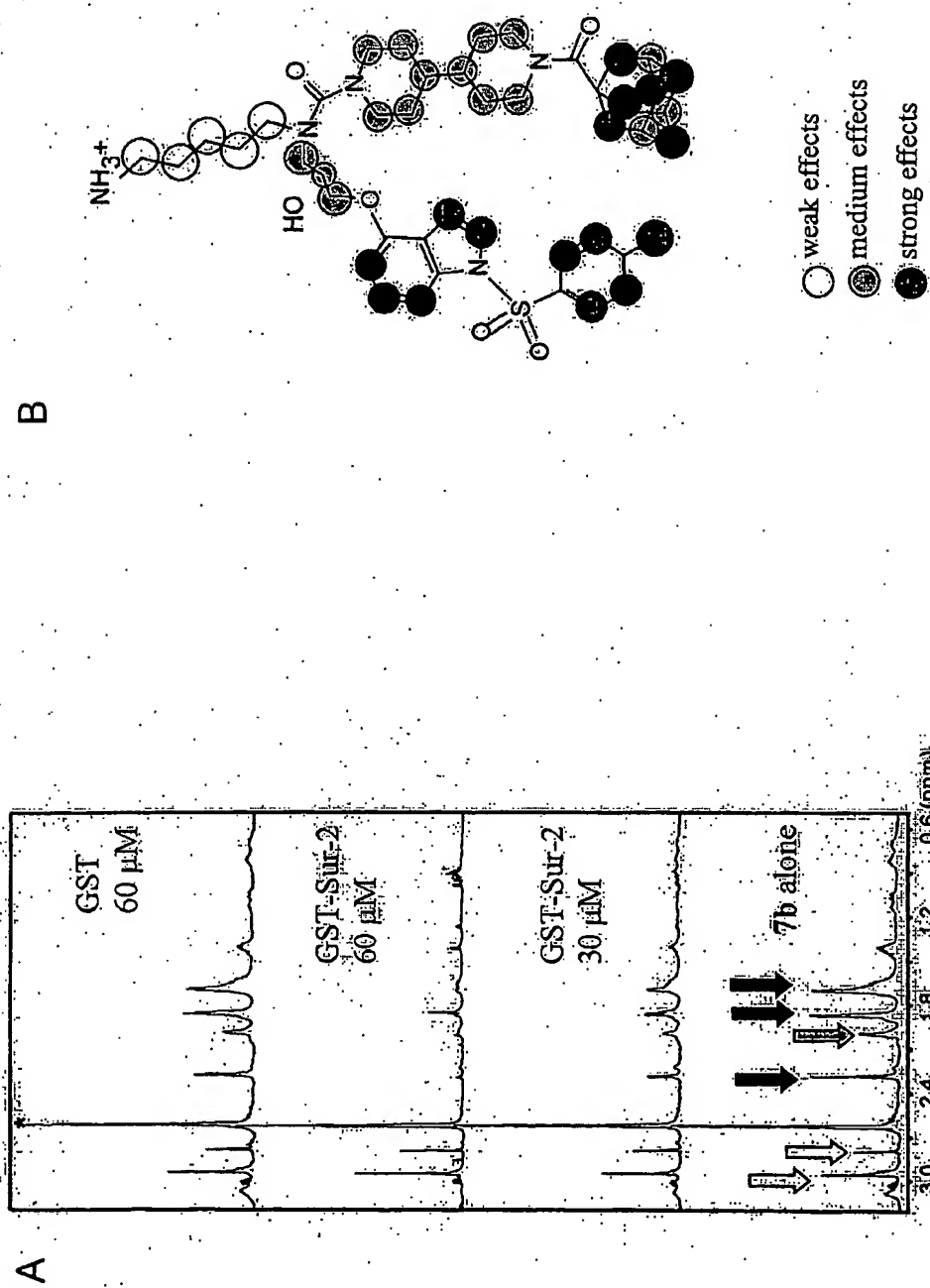
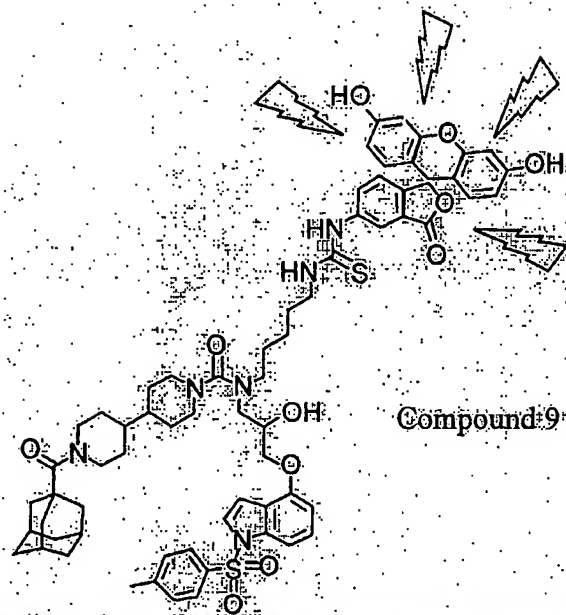


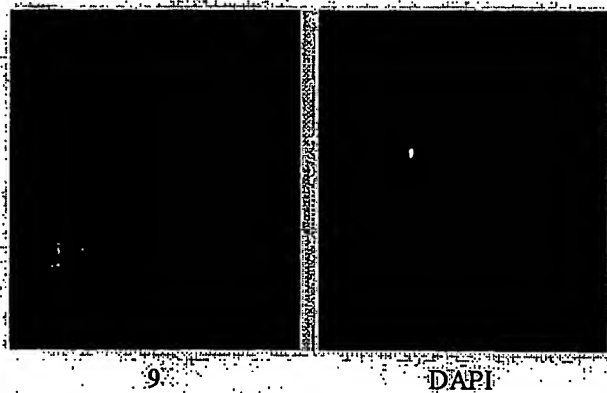
FIG. 22

35/37

A



B



C

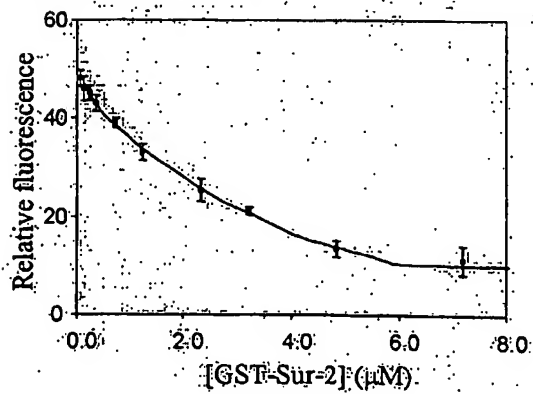


FIG. 23

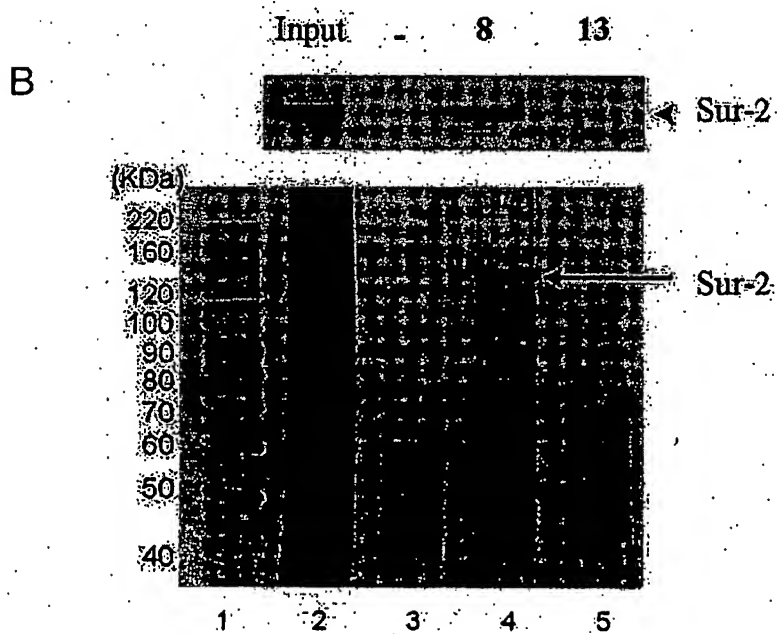
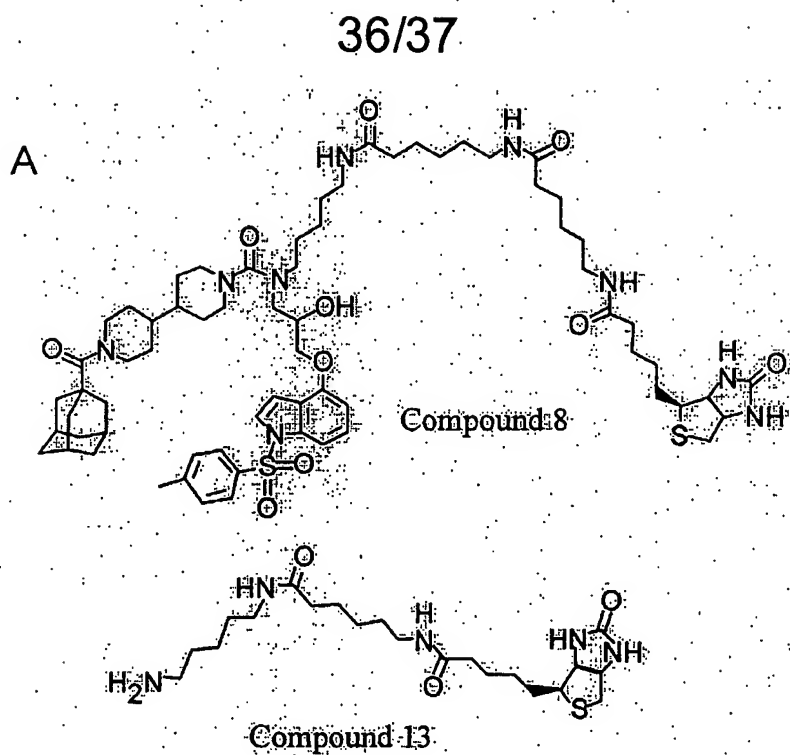
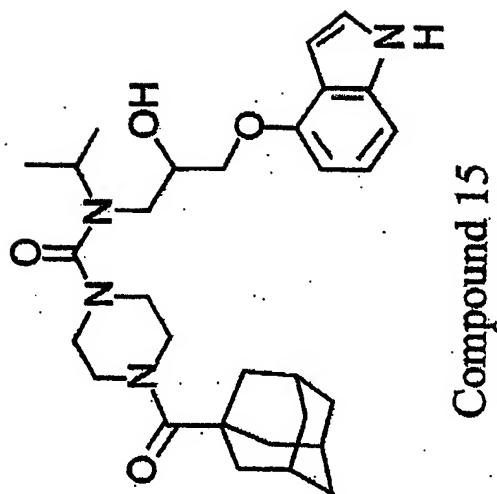
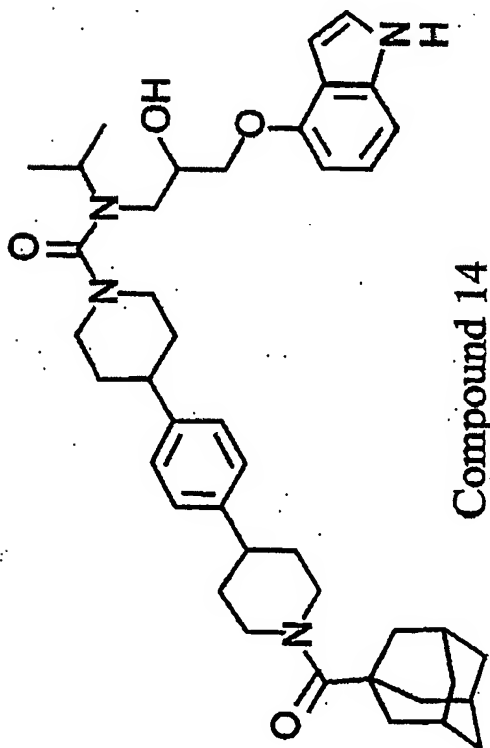


FIG. 24

37/37



Compound 15



Compound 14

FIG. 25

SEQUENCE LISTING

<110> Uesugi, Montonari
Asada, Shinichi

<120> Small Molecule Inhibitors of Her2 Expression

<130> HO-P02722WO1/10213121

<140> PCT UNKNOWN

<141> 2005-01-28

<150> US 10/770,303

<151> 2004-02-02

<160> 2

<170> PatentIn version 3.1

<210> 1

<211> 8

<212> PRT

<213> Human

<400> 1

Ser Trp Ile Ile Glu Leu Leu Glu
1 5

<210> 2

<211> 1368

<212> PRT

<213> Human

<400> 2

Met Glu Thr Gln Leu Gln Ser Ile Phe Glu Glu Val Val Lys Thr Glu
1 5 10 15

Val Ile Glu Glu Ala Phe Pro Gly Met Phe Met Asp Thr Pro Glu Asp
20 25 30

Glu Lys Thr Lys Leu Ile Ser Cys Leu Gly Ala Phe Arg Gln Phe Trp
35 40 45

Gly Gly Leu Ser Gln Glu Ser His Glu Gln Cys Ile Gln Trp Ile Val
50 55 60

Lys Phe Ile His Gly Gln His Ser Pro Lys Arg Ile Ser Phe Leu Tyr
65 70 75 80

Asp Cys Leu Ala Met Ala Val Glu Thr Gly Leu Leu Pro Pro Arg Leu
85 90 95

Val Cys Glu Ser Leu Ile Asn Ser Asp Thr Leu Glu Trp Glu Arg Thr
100 105 110

Gln Leu Trp Ala Leu Thr Phe Lys Leu Val Arg Lys Ile Ile Gly Gly
115 120 125

Val Asp Tyr Lys Gly Val Arg Asp Leu Leu Lys Val Ile Leu Glu Lys
130 135 140

Ile Leu Thr Ile Pro Asn Thr Val Ser Ser Ala Val Val Gln Gln Leu
145 150 155 160

Leu Ala Ala Arg Glu Val Ile Ala Tyr Ile Leu Glu Arg Asn Ala Cys
165 170 175

Leu Leu Pro Ala Tyr Phe Ala Val Thr Glu Ile Arg Lys Leu Tyr Pro
180 185 190

Glu Gly Lys Leu Pro His Trp Leu Leu Gly Asn Leu Val Ser Asp Phe
195 200 205

Val Asp Thr Phe Arg Pro Thr Ala Arg Ile Asn Ser Ile Cys Gly Arg
210 215 220

Cys Ser Leu Leu Pro Val Val Asn Asn Ser Gly Ala Ile Cys Asn Ser
225 230 235 240

Trp Lys Leu Asp Pro Ala Thr Leu Arg Phe Pro Leu Lys Gly Leu Leu
245 250 255

Pro Tyr Asp Lys Asp Leu Phe Glu Pro Gln Thr Ala Leu Leu Arg Tyr
260 265 270

Val Leu Glu Gln Pro Tyr Ser Arg Asp Met Val Cys Asn Met Leu Gly
275 280 285

Leu Asn Lys Gln His Lys Gln Arg Cys Pro Val Leu Glu Asp Gln Leu
290 295 300

Val Asp Leu Val Val Tyr Ala Met Glu Arg Ser Glu Thr Glu Glu Lys

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 305 | | 310 | | 315 | | 320 | | | | | | | | | |
| Phe | Asp | Asp | Gly | Gly | Thr | Ser | Gln | Leu | Leu | Trp | Gln | His | Leu | Ser | Ser |
| | | | 325 | | | | | | 330 | | | | | 335 | |
| Gln | Leu | Ile | Phe | Phe | Val | Leu | Phe | Gln | Phe | Ala | Ser | Phe | Pro | His | Met |
| | | | 340 | | | | | 345 | | | | | 350 | | |
| Val | Leu | Ser | Leu | His | Gln | Lys | Leu | Ala | Gly | Arg | Gly | Leu | Ile | Lys | Gly |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Arg | Asp | His | Leu | Met | Trp | Val | Leu | Leu | Gln | Phe | Ile | Ser | Gly | Ser | Ile |
| | 370 | | | | | 375 | | | | | 380 | | | | |
| Gln | Lys | Asn | Ala | Leu | Ala | Asp | Phe | Leu | Pro | Val | Met | Lys | Leu | Phe | Asp |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 |
| Leu | Leu | Tyr | Pro | Glu | Lys | Glu | Tyr | Ile | Pro | Val | Pro | Asp | Ile | Asn | Lys |
| | | | | 405 | | | | | 410 | | | | | 415 | |
| Pro | Gln | Ser | Thr | His | Ala | Phe | Ala | Met | Thr | Cys | Ile | Trp | Ile | His | Leu |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Asn | Arg | Lys | Ala | Gln | Asn | Asp | Asn | Ser | Lys | Leu | Gln | Ile | Pro | Ile | Pro |
| | | 435 | | | | | 440 | | | | | 445 | | | |
| His | Ser | Leu | Arg | Leu | His | His | Glu | Phe | Leu | Gln | Gln | Ser | Leu | Arg | Asn |
| | 450 | | | | | 455 | | | | | 460 | | | | |
| Lys | Ser | Leu | Gln | Met | Asn | Asp | Tyr | Lys | Ile | Ala | Leu | Leu | Cys | Asn | Ala |
| 465 | | | | | 470 | | | | | 475 | | | | | 480 |
| Tyr | Ser | Thr | Asn | Ser | Glu | Cys | Phe | Thr | Leu | Pro | Met | Gly | Ala | Leu | Val |
| | | | 485 | | | | | | 490 | | | | | 495 | |
| Glu | Thr | Ile | Tyr | Gly | Asn | Gly | Ile | Met | Arg | Ile | Pro | Leu | Pro | Gly | Thr |
| | | 500 | | | | | | 505 | | | | | | 510 | |
| Asn | Cys | Met | Ala | Ser | Gly | Ser | Ile | Thr | Pro | Leu | Pro | Met | Asn | Leu | Leu |
| | | 515 | | | | | 520 | | | | | 525 | | | |
| Asp | Ser | Leu | Thr | Val | His | Ala | Lys | Met | Ser | Leu | Ile | His | Ser | Ile | Ala |
| | 530 | | | | | 535 | | | | | 540 | | | | |

Thr Arg Val Ile Lys Leu Ala His Ala Lys Ser Ser Val Ala Leu Ala
 545 550 555 560

Pro Ala Leu Val Glu Thr Tyr Ser Arg Leu Leu Val Tyr Met Glu Ile
 565 570 575

Glu Ser Leu Gly Ile Lys Gly Phe Ile Ser Gln Leu Leu Pro Thr Val
 580 585 590

Phe Lys Ser His Ala Trp Gly Ile Leu His Thr Leu Leu Glu Met Phe
 595 600 605

Ser Tyr Arg Met His His Ile Gln Pro His Tyr Arg Val Gln Leu Leu
 610 615 620

Ser His Leu His Thr Leu Ala Ala Val Ala Gln Thr Asn Gln Asn Gln
 625 630 635 640

Leu His Leu Cys Val Glu Ser Thr Ala Leu Arg Leu Ile Thr Ala Leu
 645 650 655

Gly Ser Ser Glu Val Gln Pro Gln Phe Thr Arg Phe Leu Ser Asp Pro
 660 665 670

Lys Thr Val Leu Ser Ala Glu Ser Glu Glu Leu Asn Arg Ala Leu Ile
 675 680 685

Leu Thr Leu Ala Arg Ala Thr His Val Thr Asp Phe Phe Thr Gly Ser
 690 695 700

Asp Ser Ile Gln Gly Thr Trp Cys Lys Asp Ile Leu Gln Thr Ile Met
 705 710 715 720

Ser Phe Thr Pro His Asn Trp Ala Ser His Thr Leu Ser Cys Phe Pro
 725 730 735

Gly Pro Leu Gln Ala Phe Phe Lys Gln Asn Asn Val Pro Gln Glu Ser
 740 745 750

Arg Phe Asn Leu Lys Lys Asn Val Glu Glu Glu Tyr Arg Lys Trp Lys
 755 760 765

Ser Met Ser Asn Glu Asn Asp Ile Ile Thr His Phe Ser Met Gln Gly
 770 775 780

Ser Pro Pro Leu Phe Leu Cys Leu Leu Trp Lys Met Leu Leu Glu Thr
 785 790 795 800

Asp His Ile Asn Gln Ile Gly Tyr Arg Val Leu Glu Arg Ile Gly Ala
 805 810 815

Arg Ala Leu Val Ala His Val Arg Thr Phe Ala Asp Phe Leu Val Tyr
 820 825 830

Glu Phe Ser Thr Ser Ala Gly Gly Gln Gln Leu Asn Lys Cys Ile Glu
 835 840 845

Ile Leu Asn Asp Met Val Trp Lys Tyr Asn Ile Val Thr Leu Asp Arg
 850 855 860

Leu Ile Leu Cys Leu Ala Met Arg Ser His Glu Gly Asn Glu Ala Gln
 865 870 875 880

Val Cys Tyr Phe Ile Ile Gln Leu Leu Leu Leu Lys Pro Asn Asp Phe
 885 890 895

Arg Asn Arg Val Ser Asp Phe Val Lys Glu Asn Ser Pro Glu His Trp
 900 905 910

Leu Gln Asn Asp Trp His Thr Lys His Met Asn Tyr His Lys Lys Tyr
 915 920 925

Pro Glu Lys Leu Tyr Phe Glu Gly Leu Ala Glu Gln Val Asp Pro Pro
 930 935 940

Val Gln Ile Gln Ser Pro Tyr Leu Pro Ile Tyr Phe Gly Asn Val Cys
 945 950 955 960

Leu Arg Phe Leu Pro Val Phe Asp Ile Val Ile His Arg Phe Leu Glu
 965 970 975

Leu Leu Pro Val Ser Lys Ser Leu Glu Thr Leu Leu Asp His Leu Gly
 980 985 990

Gly Leu Tyr Lys Phe His Asp Arg Pro Val Thr Tyr Leu Tyr Asn Thr
 995 1000 1005

Leu His Tyr Tyr Glu Met His Leu Arg Asp Arg Ala Phe Leu Lys
 1010 1015 1020

Arg Lys Leu Val His Ala Ile Ile Gly Ser Leu Lys Asp Asn Arg
 1025 1030 1035

Pro Gln Gly Trp Cys Leu Ser Asp Thr Tyr Leu Lys Cys Ala Met
 1040 1045 1050

Asn Ala Arg Glu Glu Asn Pro Trp Val Pro Asp Asp Thr Tyr Tyr
 1055 1060 1065

Cys Arg Leu Ile Gly Arg Leu Val Asp Thr Met Ala Gly Lys Ser
 1070 1075 1080

Pro Gly Pro Phe Pro Asn Cys Asp Trp Arg Phe Asn Glu Phe Pro
 1085 1090 1095

Asn Pro Ala Ala His Ala Leu His Val Thr Cys Val Glu Leu Met
 1100 1105 1110

Ala Leu Ala Val Ser Gly Lys Glu Val Gly Asn Ala Leu Leu Asn
 1115 1120 1125

Val Val Leu Lys Ser Gln Pro Leu Val Pro Arg Glu Asn Ile Thr
 1130 1135 1140

Ala Trp Met Asn Ala Ile Gly Leu Ile Ile Thr Ala Leu Pro Glu
 1145 1150 1155

Pro Tyr Trp Ile Val Leu His Asp Arg Ile Val Ser Val Ile Ser
 1160 1165 1170

Ser Pro Ser Leu Thr Ser Glu Thr Glu Trp Val Gly Tyr Pro Phe
 1175 1180 1185

Arg Leu Phe Asp Phe Thr Ala Cys His Gln Ser Tyr Ser Glu Met
 1190 1195 1200

Ser Cys Ser Tyr Thr Leu Ala Leu Ala His Ala Val Trp His His

| | | | | |
|---|--|------|--|------|
| 1205 | | 1210 | | 1215 |
| Ser Ser Ile Gly Gln Leu Ser Leu Ile Pro Lys Phe / Leu Thr Glu | | | | |
| 1220 | | 1225 | | 1230 |
| Val Leu Leu Pro Ile Val Lys Thr Glu Phe Gln Leu Leu Tyr Val | | | | |
| 1235 | | 1240 | | 1245 |
| Tyr His Leu Val Gly Pro Phe Leu Gln Arg Phe Gln Gln Glu Arg | | | | |
| 1250 | | 1255 | | 1260 |
| Thr Arg Cys Met Ile Glu Ile Gly Val Ala Phe Tyr Asp Met Leu | | | | |
| 1265 | | 1270 | | 1275 |
| Leu Asn Val Asp Gln Cys Ser Thr His Leu Asn Tyr Met Asp Pro | | | | |
| 1280 | | 1285 | | 1290 |
| Ile Cys Asp Phe Leu Tyr His Met Lys Tyr Met Phe Thr Gly Asp | | | | |
| 1295 | | 1300 | | 1305 |
| Ser Val Lys Glu Gln Val Glu Lys Ile Ile Cys Asn Leu Lys Pro | | | | |
| 1310 | | 1315 | | 1320 |
| Ala Leu Lys Leu Arg Leu Arg Phe Ile Thr His Ile Ser Lys Met | | | | |
| 1325 | | 1330 | | 1335 |
| Glu Pro Ala Ala Val Pro Pro Gln Ala Met Asn Ser Gly Ser Pro | | | | |
| 1340 | | 1345 | | 1350 |
| Ala Pro Gln Ser Asn Gln Val Pro Val Ser Leu Pro Val Thr Gln | | | | |
| 1355 | | 1360 | | 1365 |

25334358.1 - 7 -

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/03349

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/445; C07D 401/04

US CL : 514/316; 546/187

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/316; 546/187

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| X | Database CAPLUS on STN (Columbus, OH, USA) No. 138:363718, 'A gene-expression inhibitor that targets an alpha-helix-mediated protein interaction', abstract, Asada et al. J. Am. Chem. Society, vol 125, pages 4992-4993 (2003). See entire document. | 1-7, 10-14, 16-19 |
| --- | | ----- |
| Y | Am. Chem. Society, vol 125, pages 4992-4993 (2003). See entire document. | 8-9, 15 |
| Y | Greene TW "Protective groups in organic synthesis" Wiley & Sons, pages 218, 222, 284 (1982) | 8-9, 15 |

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 June 2005 (22.06.2005)

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Date of mailing of the international search report

07 JUL 2005

Authorized officer

Celia Chang

Telephone No. 571-272-1600

INTERNATIONAL SEARCH REPORT

International application no.
PCT/US05/03349

Continuation of B. FIELDS SEARCHED Item 3:
CAS-structure
East/West-image